Experimental Section[5]

2,7-Dihydroxy-9-fluorenone (III).—4,4′-Dihydroxybiphenyl-2-carboxylic acid[3] (5 g) was pulverized with 10 g of anhydrous $ZnCl_2$. The mixture was heated to 200° for 10 min while stirring constantly, cooled, and decomposed in water. The precipitate was filtered and dried to yield 4.0 g (87%), mp 325–328°. Recrystallization from aqueous alcohol gave dark red crystals, mp 338° (lit.[3] mp 338°).

2,7-Dihydroxyfluorene (IV).—Compound III (2 g) was dissolved in 40 ml of hot diethylene glycol. Hydrazine hydrate (85%, 10 ml) was added, and the mixture was refluxed at 120° for 1 hr. KOH (4 g) was then added, and the temperature was raised to 200–205°. Heating was continued without a condenser for 2 hr. The reaction mixture was then cooled, treated with 30 ml of cold water, and acidified with 12 ml of concentrated HCl to precipitate IV; yield 1.7 g (90.9%), mp 262–263°. Three recrystallizations from aqueous alcohol gave white flakes, mp 269–270°[6] (lit. mp 233°[7] and 249–250°[8]).

Anal. Calcd for $C_{13}H_{10}O_2$: C, 78.78; H, 5.05. Found: C, 78.67; H, 5.12.

2,7-Di(methanesulfonyloxy)fluorene (V).—To compound IV (1 g) in 15 ml of pyridine, 2 ml of methanesulfonyl chloride was added slowly while stirring at 0°. After refrigeration overnight, the contents were poured into 100 ml of cold water. The resulting precipitate was filtered, washed with cold water, and dried. Recrystallization from a mixture of $CHCl_3$ and alcohol gave long, colorless needles, mp 166–167°, yield 1.5 g (84%).

Anal. Calcd for $C_{15}H_{14}O_6S_2$: C, 50.85; H, 3.98; S, 18.08. Found: C, 50.88; H, 3.99; S, 18.04.

2,7-Fluorenedisulfonyl chloride (VI) was made by mixing I (20 g) with PCl_5 (31 g) and heating over a steam bath for 2 hr. The mixture was decomposed in ice, filtered, washed with cold water, and dried under vacuum. It was recrystallized from ethylene dichloride, mp 227° (lit.[9] mp 225°).

Dimethyl 2,7-Fluorenedisulfonate (VII).—To sodium (0.9 g, 20 mmoles) in 100 ml of anhydrous methanol, VI (7.3 g, 10 mmoles) was added in small portions at room temperature. The precipitate was collected, and the filtrate was evaporated under vacuum. The residue was combined with the original precipitate, and the whole was stirred with 25 ml of cold water and filtered. Recrystallization from methanol gave white microneedles, mp 193–194°, yield 4.2 g (60%).

Anal. Calcd for $C_{15}H_{14}O_6S_2$: C, 50.85; H, 3.98; S, 18.08. Found: C, 50.88; H, 4.12; S, 18.29.

Animal Experiments.—Male, CAF_1/Jax mice were employed in this study. Three weeks prior to administration of the compounds, the mice received a subaxially transplant of a keratinizing squamous cell carcinoma.[10] The mice were divided into groups of three. One group consisted of control animals which received 0.2 ml ip of a 0.5% solution of methylcellulose, the vehicle for injecton, each alternate day. Compound V, at doses of 25 and 50 mg/kg and compound VII, at a dose of 500 mg/kg, were administered to three groups of animals. The treatment schedule was continued for 21 days by injecting the compounds on alternate days. On the day of drug administration, estimates of the size of the tumors were made. Tumor areas (mm^2) were plotted against the number of days. Animal weights were also recorded on the same days and plotted. After termination of the treatment, tumor sizes were estimated for another 14 days. The animals were further observed for a period of 5 weeks to determine the number of survivals among treated and control animals.

Acknowledgment.—The author wishes to express his sincere appreciation to Dr. F. E. Ray for helpful discussions and to the Sloan-Kettering Institute for Cancer Research, New York, N. Y., for screening results. Thanks are due the American Cancer Society for an allotment from the University of Florida Institutional Grant IN-62E and 66-7.

(5) Melting points were determined on a Thomas–Hoover capillary apparatus and are recorded as obtained. Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y.

(6) Discrepancies in the melting point of compound IV seems to be due to differences in purity. The purity of compound III is also an important factor in the final purity of IV: lower melting points resulted from the use of crude III.

(7) C. D. Nenitzescu and M. Avram, *Acad. Rep. Populare Romine, Studii Cercetari Chim.*, **4**, 57 (1956).

(8) A. Barnes and R. W. Faessinger, *J. Org. Chem.*, **26**, 4544 (1961).

(9) C. Courtot and R. Geoffroy, *Compt. Rend.*, **178**, 2259 (1924).

(10) Line a, stomach carcinoma originally obtained from the animal supply and research units of the British Empire Cancer Campaign.

New Alkylating Agents Derived from Diaziridine[1]

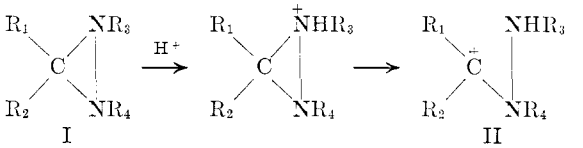
CSABA SZANTAY, Z. F. CHMIELEWICZ, AND T. J. BARDOS[2]

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14214

Received July 11, 1966

Several of the best known biological alkylating agents that were found effective as antineoplastic drugs[3] and/or insect chemosterilants[4] contain aziridine (ethylenimine) rings as their reactive functional groups. In this laboratory there has been particular interest in the synthesis and study of a series of ring-substituted bis(1-aziridinyl)phosphinylurethans ("dual antagonists")[5] as well as such other ring-substituted aziridine derivatives[6] that might be capable of undergoing SN1-type reactions under biological conditions.[7]

Diaziridines (I) were shown recently to undergo acid hydrolysis by an SN1 mechanism[8] which must involve ring opening with the formation of a carbonium ion (II). It was thought that diaziridines might act as



biological alkylating agents if their ring-opening reaction is sufficiently "activated" by electron-attracting R_3 substituents similar to those that were proved effective in the case of the aziridines (C=O, P→O, P→S, etc.). It seemed, therefore, of interest to prepare some diaziridinyl analogs of the ring-substituted bis(1-aziridinyl)phosphinylurethan[5] and tris(1-aziridinyl)phosphine oxide[3] series of known chemotherapeutic agents.

While on the basis of analogy[6] and rationale,[7] the corresponding derivatives of 3,3-dialkyldiaziridines would seem to be the most desirable ones; unfortunately, such diaziridine rings (derived from ketones) are known to be unstable toward acylating reagents.[9,10]

(1) This investigation was supported by Grant CA-06695 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

(2) To whom inquiries should be directed.

(3) See, *e.g.*, D. A. Karnofsky and F. Bergel in "Chemotherapy of Cancer," P. L. Plattner, Ed., Elsevier Publishing Co., New York, N. Y., 1964, pp 3–18 and 21–31.

(4) A. B. Borkovec, *Science*, **137**, 1034 (1962).

(5) (a) T. J. Bardos, A. K. Barua, Z. F. Chmielewicz, G. E. Crevar, J. P. Dailey, S. Divald, and Z. B. Papanastassiou, *J. Pharm. Sci.*, **54**, 187 (1965); (b) T. J. Bardos, Z. F. Chmielewicz, and C. K. Navada, *ibid.*, **54**, 399 (1965).

(6) Z. F. Chmielewicz, T. J. Bardos, A. Segaloff, A. Munson, and J. L. Ambrus, *Proc. Am. Assoc. Cancer Res., Denver, Colo., May, 1966*, **7**, 14 (1966).

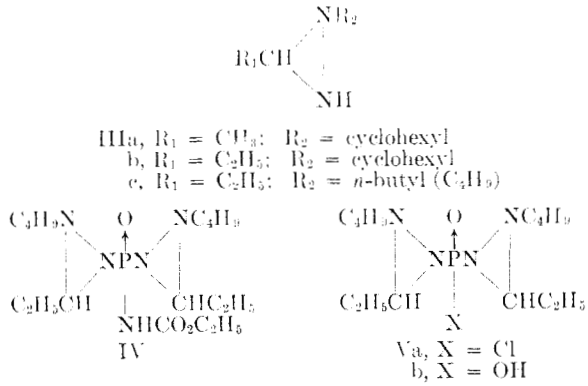
(7) (a) T. J. Bardos, *Biochem. Pharmacol.*, **11**, 256 (1962); (b) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).

(8) C. Szantay and E. Schmitz, *Chem. Ber.*, **95**, 1759 (1962).

(9) E. Schmitz, in *Advan. Heterocyclic Chem.*, **2**, 83 (1963).

(10) H. J. Abendroth, *Angew. Chem.*, **73**, 67 (1961).

Therefore, only diaziridines with one C-alkyl substituent (IIIa–c) could be considered as starting materials for the synthesis of the desired compounds. These diaziridines were prepared by the reaction of hydroxylamine-O-sulfonic acid with the Schiff base derived from the appropriate aldehyde and alkylamine, using a modification of the methods described in the literature.[9–11]



These diaziridines (III) were treated in benzene solution with dichlorophosphinylurethan[12] in the presence of triethylamine, in the manner described for the preparation of the corresponding aziridine derivatives.[5,12] It was found, however, that this reaction proceeds much more slowly in the case of the diaziridines, and it could not be carried to completion at room temperature with either of the two N-cyclohexyl derivatives, IIIa or IIIb. On warming the reaction mixtures above 40°, decomposition products were formed. More successful was the reaction of the N-*n*-butyl diaziridine IIIc which, at room temperature after an extended reaction time, gave the desired product IV. The latter was identified by elemental analysis, by infrared and nmr spectra, and by the characteristic reaction of diaziridine rings with hydrogen iodide.[9] This reagent is oxidized by IV to iodine in a quantitative manner (corresponding to 2 equiv of diaziridine/mole) but at a much slower rate than by IIIa–c or other alkyldiaziridines.

Reaction of IIIc with phosphorus oxychloride resulted in the replacement of only two chlorine atoms to give Va. On dissolving Va in aqueous alcohol, it was immediately hydrolyzed (with the release of 1 equiv of HCl), presumably, to Vb.

Results of a kinetic study of the hydrolysis of the diaziridine rings in 1 *N* sulfuric acid–50% aqueous ethanol solution at 22° are shown in Figure 1. The rate of disappearance of the diaziridine rings was followed by iodometric titration. This rate was slower for IV than for the corresponding "free" diaziridine IIIc, which had a rate constant, k, of 0.17×10^{-5} sec^{-1}. At the same time, the oxidation rate of hydrogen iodide by IV increased relatively rapidly through the first 12 hr of hydrolysis (see Figure 2) indicating the formation of an intermediate diaziridine derivative; this, rather than IV itself, may be the species primarily undergoing the ring-opening reaction. Such intermediate diaziridine derivatives might arise by the splitting of one of the P–N bonds of IV to give either

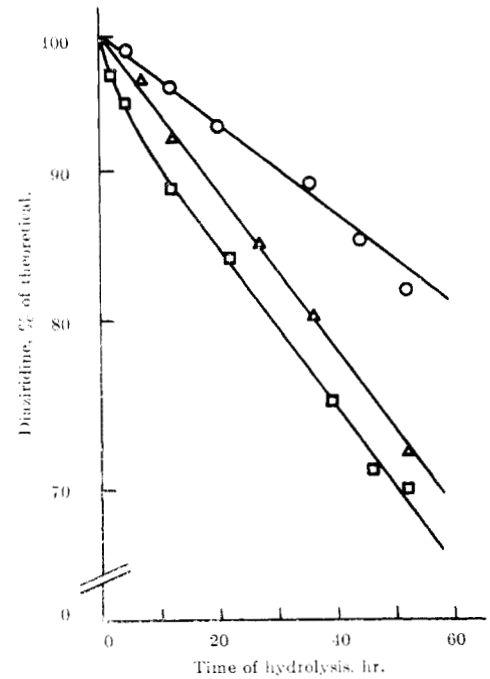


Figure 1.—Semilogarithmic plot of the rates of ring opening in acid hydrolysis (1 *N* H_2SO_4–50% aqueous ethanol, 22°) of diaziridine derivatives: ○ = IV, △ = IIIc, □ = Va. The remaining amounts of intact diaziridine rings, as determined by iodometric titration (using the 1-hr titration values, $I_2^{60'}$, *cf.* Figure 2) and expressed as percentages of the theoretical value for the given compound, are plotted against the duration of hydrolysis of the samples.

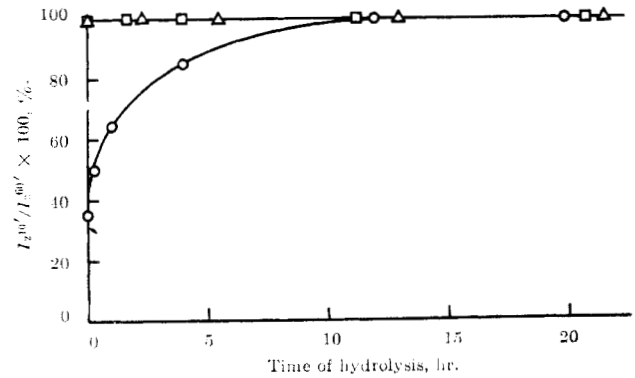


Figure 2.—Effect of acid hydrolysis on the rates of reaction of the diaziridine derivatives with HI: ○ = IV, △ = IIIc, □ = Va. The relative amounts of iodine liberated during the first 10 min ($I_2^{10'}$) as percentages of the total amounts of iodine liberated during 1 hr ($I_2^{60'}$) following the addition of HI to the samples are plotted against the times of acid hydrolysis (*cf.* Figure 1) of the samples prior to the addition of HI.

the "free" diaziridine IIIc or a carbamate-free hydrolysis product similar to Vb. Since the oxidation rate of hydrogen iodide by Va was found to be as fast as by IIIc, it is conceivable that Vb is formed as the first intermediate in the acid hydrolysis of IV.

Both IV and Va showed some, but very low, alkylating activity in the (usually S$_N$2-type) reaction with the nucleophilic reagent 4-(*p*-nitrobenzyl)pyridine at 80° under the previously described conditions.[7b] In this reaction, they showed approximately the same alkylating activity as the 2,2,3,3-tetramethylaziridine analog of IV ($k_{80'} = 0.4 \times 10^{-3}$ min^{-1}) which has the lowest activity in the aziridine series.[5b]

(11) C. J. Paget and C. S. Davis, *J. Med. Chem.*, **7**, 626 (1964).
(12) Z. B. Papanastassiou and T. J. Bardos, *ibid.*, **5**, 1000 (1962).

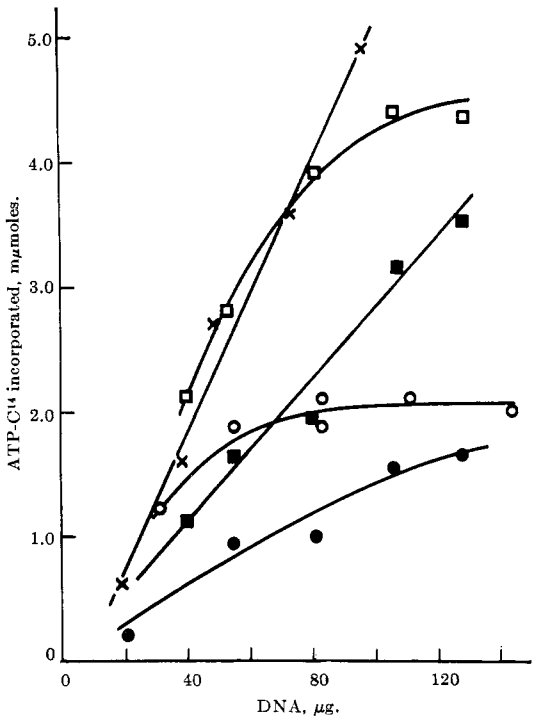


Figure 3.—Effect of diaziridine derivatives on the priming activity of calf thymus DNA in a DNA-dependent, RNA-polymerase system: × = DNA control (16 and 60 hr), □ = DNA incubated with IV for 16 hr, ■ = same, for 60 hr, ○ = DNA incubared with Va for 16 hr, ● = same, for 60 hr. See details in Experimental Section.

Biological Activity.—Due to the poor solubility of IV and the instability of Va, some difficulties were encountered in the initial *in vivo* testing of these compounds against transplanted animal tumors, and the results obtained, so far, are inconclusive. Both compounds showed moderate cytotoxicities against a Sarcoma 180 cell line,[13] giving 50% inhibition in the 10–25-μg/ml concentration range and full inhibition at 100 μg/ml.

In vitro experiments using a DNA-dependent RNA–polymerase from *Micrococcus lysodeikticus*,[14] indicate that both IV and Va may be capable of alkylating DNA under physiologic conditions. Calf thymus DNA, on incubation with the compounds at 37°, showed significant decrease in its ability to serve as "template" in the RNA–polymerase system (see Figure 3). This effect is qualitatively similar to that shown under identical experimental conditions by the biologically active aziridine derivatives and nitrogen mustard.[15] By this assay, Va was considerably more active than IV (in line with its greater reactivity in the SN1-type ring-opening hydrolysis) and was comparable in activity to some aziridine derivatives[5a,16] that had shown significant antitumor effects at relatively high nontoxic dose levels.

(13) G. E. Foley and B. P. Drolet, *Proc. Soc. Exptl. Biol. Med.*, **92**, 347 (1956).

(14) T. Nakamoto, C. F. Fox, and S. B. Weiss, *J. Biol. Chem.*, **239**, 167 (1966).

(15) A study on the effects of nitrogen mustard and of a series of biologically active aziridine derivatives on the priming activity of DNA in this enzyme system, in correlation with changes in the physicochemical properties of the macromolecule, was reported at the Annual Meeting of the American Association for Cancer Research, Denver, Colo., May 26–28, 1966, and will be published in detail.

(16) Z. F. Chmielewicz, R. J. Fiel, T. J. Bardos, and J. L. Ambrus, to be published.

Experimental Section[17]

1-*n*-Butyl-3-ethyldiaziridine (IIIc).—To 150 g (2.05 moles) of butylamine in 300 ml of ether was added dropwise, 120 g (2.08 moles) of propionaldehyde in 200 ml of ether. The addition was carried out in the course of 1 hr under continuous (magnetic) stirring and cooling below 10°. After 1 hr, the aqueous layer (31 ml) was separated, the ether solution was dried over K_2CO_3, and, after evaporation of the solvent, the residue was distilled under reduced pressure; yield 187 g (81%) of N-propylidenebutylamine,[18] bp 35° (20 mm). To this Schiff base (160 g, 1.42 moles), 400 ml of water, and 310 g (3.05 moles) of triethylamine was added under cooling (ice–salt bath) 160 g (1.47 moles) of hydroxylamine-O-sulfonic acid during 30 min while keeping the temperature under 12°. Stirring was continued at 0–10° for 2 additional hr, and the aqueous layer was separated and extracted with several portions of ether (total volume 500 ml). After drying, the ether was evaporated *in vacuo*, and the residue was distilled at 16 mm to give fraction A, bp <69° (30.9 g), and fraction B, bp 69–73° (170 g). Iodometric titration[9] indicated that the former contained 55%, and the latter 80% of the calculated diazidiridine. Fraction A was redistilled, and the fraction collected between 66 and 69° (20 g, 75% diaziridine) was combined with fraction B. To this was added, in several portions under cooling, 63 g of anhydrous oxalic acid in 450 ml of methanol. After allowing it to crystallize in the refrigerator overnight, the oxalate salt of the diaziridine was separated by filtration (92 g). The mother liquor was concentrated to yield several more crops of the oxalate salt which were combined with the first crop and recrystallized from 270 ml of methanol; yield 92 g (29.8%) of pure IIIc oxalate (iodometrically[9] determined diaziridine content, 100%). The salt has no sharp melting point and started decomposing at 108°.

Anal. Calcd for $C_9H_{18}N_2O_4$: C, 49.60; H, 8.30; N, 12.82. Found: C, 49.72; H, 8.25; N, 12.68.

The above oxalate salt was suspended in water (200 ml), a layer of ether was added, and, under stirring and cooling, a concentrated aqueous solution of 36 g of NaOH was added dropwise, while keeping the temperature below 15°. The precipitated sodium oxalate was filtered and washed with ether, and the combined ether layer and washings were dried with K_2CO_3 and evaporated. The residue, 49.5 g (27.5%), was essentially pure (99.8% by iodometric titration) 1-*n*-butyl-3-ethyldiaziridine with nmr absorption at 2.0–2.5 (3 H, ring CH and NCH_2), 1.2–1.7 (6 H, 3 methylene groups), and 0.8–1.2 (6 H, 2 methyl groups) ppm.

The known[9] IIIa and IIIb were prepared analogously in yields of 45 and 52%, respectively.

Ethyl [Bis(1-*n*-butyl-3-ethyldiaziridinyl-2-)phosphinyl]carbamate (IV).—Freshly distilled dichlorocyanatophosphine oxide[12] (9.6 g, 0.06 mole) was converted to the ethyl carbamate derivative in the previously described[5,12] manner. To the obtained toluene solution (160 ml) was added dropwise under cooling and stirring, a solution of IIIc (15.4 g, 0.12 mole) and triethylamine (12.2 g, 0.12 mole) in dry toluene (100 ml). After 12 days at room temperature, the precipitated triethylamine hydrochloride was separated by filtration (16.5 g, 93%), and the filtrate was concentrated *in vacuo*. The residue was triturated in *n*-pentane (150 ml), and the solution obtained was decanted from resinous material. The pentane solution was stored at −20° for 2 weeks, during which time 1.5 g of the product (IV) crystallized; mp 54–55°, unchanged after recrystallization from toluene–pentane. Iodometric titration gave the calculated diaziridine content indicating that the product was pure; ν_{max}^{KBr} (cm^{-1}) 3120 (w) (NH), 3000 (s) (CH), 1740 (s) (C=O), 1460 (s) (CH_2), 1480 (w) (CH_3), 1290 (s) (P=O), 1250 (s) (CN), 1200 (s) (CO), 1060 (w), 1090 (m), 975 (w), 925 (m), 850 (m), 780 (m). These spectral bands are generally similar to those of the analogous aziridine compounds, *i.e.*, bis(1-aziridinyl)phosphinylcarbamates.[5] The nmr spectrum shows the quartet of the urethan CH_2 protons (δ 4.2), in addition to multiplets corresponding to the ring CH (δ 2.1), alkyl CH_2 and CH_3 protons.

Anal. Calcd for $C_{17}H_{36}N_5O_3P$: C, 52.49; H, 9.26; N, 17.99. Found: C, 52.43; H, 9.31; N, 17.89.

The mother liquor from IV was evaporated to give 8 g of an oily residue which appeared to be identical with the crystalline

(17) Microanalyses by Galbraith Laboratories, Knoxville, Tenn. The nmr spectra were determined (Varian A-60) in CCl_4 solution, with tetramethylsilane as internal standard.

(18) K. B. Everard and L. E. Sutton, *J. Chem. Soc.*, 2319 (1949).

material in spectral and chemical properties but could not be induced to yield more crystals.

Bis(1-*n*-butyl-3-ethyldiaziridinyl-2-)phosphinic Chloride (Va).—To a solution of IIIc (10 g, 0.0785 mole) and triethylamine (15 g, 0.146 mole) in toluene (300 ml), cooled to −15°, was added $POCl_3$ (6 g, 3.7 ml, 0.04 mole) in toluene (30 ml). After 3 days at room temperature with exclusion of air, the precipitated triethylamine hydrochloride was filtered (10 g, 94%), and the solvent was evaporated *in vacuo*. The residue, a very hygroscopic, oily substance, was completely soluble in pentane. In 50% aqueous ethanol, it was immediately hydrolyzed (presumably to Vb) with lowering of the pH to 4 and quantitative liberation of 1 equiv of chloride ion (based on the molecular weight calculated for Va), as determined by titration with $AgNO_3$. Attempts to isolate Vb from the aqueous solution led to partially polymerized syrupy material. Iodometric determination of the diaziridine groups[9] also gave results in agreement with structure Va; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 3000 (s) (CH), 1460 (s) (CH_2), 1480 (m) (CH_3), 1290 (sh) (P=O), 1250–1210 (s) (CN), 1060 (w), 975–920 (w), 720 (s). Due to the instability of the compound, an elemental analysis was not performed.

Hydrolysis Rate Studies.—The compound (0.1 g) was dissolved in a mixture of ethanol (10 ml) and 2 *N* aqueous H_2SO_4 (10 ml), and the solution was kept in a thermostat at 22°. After the specified period, 2 ml of a 20% aqueous KI was added to the sample, and the iodine was titrated after 10 min, then again after 1 hr, with 0.1 *N* $Na_2S_2O_3$.

Effects on the Activity of DNA as "Template" in a DNA-Dependent RNA–Polymerase System.[15]—A solution of calf thymus DNA, 1.5 mg/ml in 0.1 *M* phosphate buffer, pH 5.3, was incubated with 15 μmoles of each of the compounds at 37° for 16 and 60 hr. The DNA was precipitated by the addition of ethanol and isolated by centrifugation. Control samples of DNA (incubated in the same buffer, without the compounds) were prepared in an identical manner. The "template" activities of the treated and control DNA samples were compared in a DNA-dependent RNA–polymerase system, using a modification[16] of the assay described by Nakamoto, *et al.*[14] The reaction mixture, 0.5 ml, contained varying amounts of DNA, RNA–polymerase (15–30 units),[14] 0.4 μmole each of the triphosphates of uridine, cytidine, and guanosine, 0.2 μmole of 8-^{14}C-adenosine triphosphate (2.5×10^3 counts/min), 0.8 μmole of spermidine phosphate, 1.25 μmoles of $MnCl_2$, and 50 μmoles of Tris buffer, pH 7.5. After incubation with agitation for 30 min at 30°, the reaction was terminated by immersion in ice and addition of 0.1 ml of 50% trichloroacetic acid (TCA). Carrier ribonucleic acid, 0.1 ml (2.0 mg/ml), was added, and the final volume was brought to 1.0 ml by addition of 5% TCA. The solids were collected by centrifugation, washed twice with 5% TCA, and dissolved in formic acid. Aliquots were plated on stainless steel planchets, diluted with water, and dried, and the radioactivity was determined in a gas-flow counter. Incorporation of ^{14}C-ATP (millimicromoles) was plotted *vs.* the concentration of DNA (micrograms per tube, on the basis of optical density at 260 mμ)[19] to give the comparative-activity curves shown in Figure 3.

Acknowledgment.—The authors wish to express their thanks to Mr. James Baker and Mrs. Catherine Kavai for their technical assistance in this work.

(19) T. J. Bardos, J. L. Ambrus, Z. F. Chmielewicz, A. G. Penny, and C. M. Ambrus, *Cancer Res.*, **25**, 1238 (1965).

Aminomethylation and Hydroxymethylation of Purine-6(1H)-thione and 6-Alkylthiopurines[1a]

C. P. BRYANT[1b] AND R. E. HARMON[1c]

Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001

Received February 15, 1966
Revised Manuscript Received August 25, 1966

An extensive investigation of the anticancer activity of S-substituted derivatives of purine-6(1H)-thione would be of great interest because numerous investigators[2] have prepared alkylthiopurines and found that they showed activity against Adenocarcinoma 755[3] and Sarcoma 180[4] comparable to that of purine-6(1H)-thione. Grillot, *et al.*,[5] had noted earlier that Mannich reactions involving thiophenol as well as substituted thiophenols gave S-substituted thiophenols instead of C-alkylated products. This observation prompted us to study the reaction of purine-6(1H)-thione with aqueous formaldehyde and a secondary amine such as piperidine or morpholine. These reactions gave good yields of products, with sharp melting points, which gave one spot on thin layer chromatograms. The ultraviolet spectra of these compounds showed λ_{max} 328 mμ (ϵ_{max} 21,400) for the morpholine derivative (**11**) and λ_{max} 327 mμ (ϵ_{max} 18,000) for the piperidino derivative (**12**). These ultraviolet spectra lead us to believe that substitution was not occurring at the thiono group of purine-6(1H)-thione. Burckhalter and Dill[6] treated theophylline with aqueous formaldehyde and secondary amines to give 7-(dialkylaminomethyl)theophyllines (caffeine derivatives). However, the ultraviolet spectra of the products obtained from purine-6(1H)-thione did not show the bathochromic shift expected for a 7-substituted purine.[7] The fact that the products showed absorptions very similar to purine-6(1H)-thione, λ_{max} 328 mμ (ϵ_{max} 16,800), indicated that substitution was occurring in either the 1, 8, or 9 positions. Bredereck and co-workers[8] found that caffeine was hydroxymethylated in the 8 position by aqueous formaldehyde.

Compound **13** was obtained when morpholinomethylpurine-6(1H)-thione in aqueous ethyl alcohol containing sodium hydroxide was treated with *n*-propyl bromide at 45°. Compound **13** is identical with the product formed from 6-(*n*-propylthio)purine, aqueous formaldehyde, and morpholine. When **13** was treated with dimedon in ethyl alcohol at room temperature, a solid formed which proved to be the dimedon–formaldehyde adduct. Evaporation of the filtrate, which remained after the adduct was collected, gave 6-(*n*-propylthio)purine. This evidence, in combination with the ultraviolet spectra, thin layer, and paper chromatographic data, indicates that purine-6(1H)-thione undergoes the Mannich reaction at the 9 position.

The failure of 9-cyclopentyl-9H-purine-6(1H)-thione to morpholinomethylate when treated with aqueous formaldehyde and morpholine under the usual condi-

(1) (a) This work was supported in part by a Research Grant from the Michigan Cancer Foundation and a National Cancer Institute Grant, CA-06140, U. S. Public Health Service, and was presented at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 12–17, 1965, p 8P. (b) Abstracted in part from the thesis of C. P. Bryant, submitted in partial fulfillment of the Master's of Arts Degree. (c) To whom inquiries should be addressed.

(2) (a) C. G. Skinner, R. G. Ham, D. C. Fitzgerald, Jr., R. E. Eakin, and W. Shive, *J. Org. Chem.*, **21**, 1300 (1956); (b) T. P. Johnson, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958); (c) C. G. Skinner, W. Shive, R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *ibid.*, **78**, 5097 (1956); (d) L. R. Lewis, C. W. Noell, A. G. Beaman, and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 607 (1962); (e) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Org. Chem.*, **24**, 259 (1959).

(3) H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, Jr., *Proc. Am. Assoc. Cancer Res.*, **2**, 346 (1958).

(4) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.*, **18**, 445 (1958).

(5) G. F. Grillot, H. R. Felton, B. R. Garrett, H. Greenberg, R. Green, R. Clementi, and M. Moskowitz, *J. Am. Chem. Soc.*, **76**, 3969 (1954).

(6) J. H. Burckhalter and D. R. Dill, *J. Org. Chem.*, **24**, 562 (1959).

(7) G. B. Elion in The Ciba Foundation Symposium, Chemistry and Biology of Purines, G. E. W. Wolstenholm and C. M. O'Connor, Ed., Little, Brown, and Co., Boston, Mass., 1957, p 39.

(8) H. Bredereck, E. Siegel, and G. Fohlisch, *Ber.*, **95**, 103 (1962).