dried over anhydrous magnesium sulfate, then concentrated *in vacuo* to a brown sirup. This sirup was dissolved in 50 ml. of hot acetone and the solution allowed to stand overnight at room temperature. The white, powdery solid which had separated was collected and washed with acetone; yield 2.3 g. (45%); m.p. 178–182°. A portion of this solid was dissolved in 20% hydrochloric acid and neutralized with a saturated aqueous solution of sodium acetate causing a white solid to separate. The filtrate upon partial concentration *in vacuo* yielded an analytical sample of VII, m.p. 185–188°; $\lambda_{max(\mu)}^{Nujol}$ 6.21, 6.30, 6.40 (NH_3^+, CO_2^-, aryl); 6.63 (aryl, NH_3^+); 7.06 (CO_2^-); 12.8 (*m*-disubstituted benzene); 13.5 (C—Cl). The compound (XI) traveled as a single ninhydrin and ultraviolet absorbing positive spot (R_f 0.84) in solvent system A.[11]

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O_2$: C, 51.2; H, 5.92, Cl, 23.2; N, 9.18. Found: C 51.1; H, 6.06, Cl, 23.3; N, 9.20.

*3-{4-[Bis(2-chloroethyl)amino]-3-chlorophenyl}-*DL-*alanine* (IV). To a stirred suspension of 3.05 g. (0.010 mole) of 3-{*p*-[bis-(2-chloroethyl)amino]phenyl}-DL-alanine (I) in 30 ml. of glacial acetic acid heated to 50° was added dropwise in about 2–3 min. 0.85 ml. (0.010 mole) of sulfuryl chloride, maintaining the temperature between 50–55° by external cooling. Ten minutes after all the sulfuryl chloride had been added, the solution was concentrated *in vacuo* to 5 ml., diluted with 10 ml. of water, and neutralized with saturated sodium acetate solution. The gum which separated was triturated with water until it solidified. Solution of the solid in 25 ml. of methanol and addition of water caused a dark gum to separate. After removal of this gum, further addition of water yielded a granular solid upon chilling; yield 1.4 g. (41%), m.p. 166–173°; $\lambda_{max(\mu)}^{Nujol}$ 4.80 (NH_3^+); 6.09 (amino acid I); 6.65 (amino acid II); 6.31, 7.13 (CO_2^-). The compound (IV) traveled as a single ninhydrin positive spot (R_f 0.72) in solvent system C.[11] The starting material (I) traveled nearly the same (R_f 0.68) in that system.

Anal. Calcd. for $C_{13}H_{17}Cl_3N_2O_2$: C, 45.9; H, 5.04; Cl, 31.3. Found: C, 45.5; H, 5.18, Cl, 31.3. An analysis for ionic chloride, carried out at 0°, gave 0.45%.

*3-{m-[Bis(2-chloroethyl)amino]phenyl}-N-formyl-*DL-*alanine* (VI). To a solution of 0.60 g (2.0 mmole) of 3-{*m*-[bis-(2-chloroethyl)amino]phenyl}-DL-alanine (V) in 4 ml. of 90% formic acid was added 1.2 ml. of acetic anhydride. The red colored solution was warmed at 50–55° for 30 min. After cooling, the reaction mixture was diluted with 20 ml. of water and the product slowly crystallized; yield; 0.61 g. (91%), m.p. 145–150°. A sample for analysis was prepared by recrystallization from absolute ethanol, m.p. 151–152°; $\lambda_{max(\mu)}^{Nujol}$ 2.99 (NH); 5.80 (acid C=O); 6.19 (amide C=O); 6.64 (amide NH); 12.9 (*m*-disubstituted benzene). This compound (VI) traveled as a single spot (R_f 0.93) in solvent system A[11] and had an R_f of 0.71 in solvent system B. The chromatograms were ninhydrin negative at 200 γ while the starting amino acid (V) was detectable at 5 γ using ninhydrin reagent, thus showing less than 3% of (V) could have been present. Compound V had an R_f of 0.85 in solvent system A and R_f of 0.48 in solvent system B.[11]

Anal. Calcd. for $C_{14}H_{18}Cl_2N_2O_3$: C, 50.4; H, 5.42; Cl, 21.3; N, 8.40. Found: C, 50.5; H, 5.44; Cl, 21.0; N, 8.34.

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MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, YALE UNIVERSITY]

Synthesis of Some 8-Purinyl Nitrogen Mustards[1]

SHIH-HSI CHU, JACK E. HARRIS,[2] AND HENRY G. MAUTNER

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As part of a program concerned with the preparation of nitrogen mustards intended to exhibit biological specificity, the 8-bis(β-chloroethyl)amino derivatives of xanthine, hypoxanthine, and adenine were synthesized.

In recent years large numbers of nitrogen mustards, *i.e.*, compounds containing the bis-(β-chloroethyl)amino grouping, have been synthesized as potential antitumor agents. In the hope of increasing the biological specificity of these compounds the mustard group has been attached to various carrier molecules such as antimalarial drugs,[3] amino acids[4,5] steroids,[6] and carbohydrates,[7] to name only a few.

In view of the hypothesis that double armed mustards exert their carcinostatic effects by reacting with the phosphate groups of nucleic acids, thus causing cross-linking between adjoining double helices,[8] it seemed of interest to synthesize compounds which would facilitate the likelihood of such cross-linking occurring.

This problem was approached in two ways, (a) by synthesizing 8,8′-bispurines,[9] and (b) by synthesizing the 8-purinyl nitrogen mustards described in this communication. Incorporation of 8,8′-bispurines (or their deoxyribonucleosides) in neighboring deoxyribonucleic acid double helices might result in direct crosslinking, while incorporation of

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(2) Present address, Monsanto Chemical Co., Everett, Mass.

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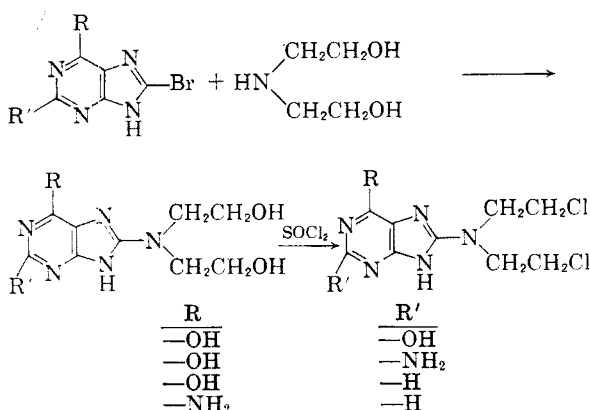
8-purinyl mustards might result in subsequent cross-linking by chemical reaction. The 8-position was chosen since bulky and reactive substituents in the 2- and 6-positions of purines would interfere with the hydrogen bonding required to form the Watson-Crick double helix, while the 9-position is required for deoxyribonucleosidation. Recently the synthesis of 5-bis(β-chloroethyl)aminouracil, a compound with considerable antitumor activity,[10] has been reported. Here again the mustard group is attached in a position where interference with hydrogen-bonding or with ribonucleosidation is minimized. It would seem reasonable to assume that whenever reactive groupings are attached to metabolites to form "toxophoric antimetabolites," these groups should not replace substituents required for attachment to the receptor site but should be located in their vicinity.[11,12]

The following synthetic scheme was utilized:



The reaction of 8-bromopurines with diethanolamine proceeded smoothly in boiling ethylene glycol monomethyl ether under nitrogen. The 8-chloro-compounds were considerably less reactive. Reaction of the bis(β-hydroxyethyl)amino compounds with thionyl chloride then yielded the desired mustards in the form of hydrochlorides. The only exception proved to be 8-bis(β-hydroxyethyl)aminoguanine which, on being treated with thionyl chloride, did not yield the desired guanine-mustard. Considerable difficulties were encountered in obtaining analytical samples of all the bis(β-chloroethyl)amino compounds reported on herein

Biological work with these compounds is now in progress and will be described elsewhere.

EXPERIMENTAL

8-Bromopurines. 8-Bromoadenine and 8-bromohypoxanthine were prepared by the method of Kruger[13] and 8-bromoguanine and 8-bromoxanthine by the method of Fischer and Ruse.[14]

8-Bis(β-hydroxyethyl)aminoadenine. A mixture of 5.3 g. (0.0248 mole) of 8-bromoadenine and 25 g. of diethanolamine in 50 ml. of ethylene glycol monomethyl ether was stirred mechanically and heated to boiling over a period of 20 hr., while being kept under nitrogen. The solvent was removed under vacuum and the residue extracted five times with 25-ml. portions of hot acetone. Ethanol was added to the residual oil, and the resulting solid removed by filtration. Concentration of the combined washings and filtrate resulted in the separation of more material, the combined yield being 3.0 g. (50%). The product was recrystallized from hot water to yield a white powder melting at 245–249°.[15] Ultraviolet spectrum. *p*H 1, λ_{max} 300 mμ, ϵ_{max} 16,700.

Anal.[16] Calcd. for $C_9H_{14}N_6O_2$: C, 45.36; H, 5.92; N, 35.27. Found: C, 45.58; H, 6.01; N, 35.13.

8-Bis(β-hydroxyethyl)aminohypoxanthine. This material was obtained from 8-bromohypoxanthine and diethanolamine in 49% yield and recrystallized from water to yield delicate white needles m.p. 250–255° dec. Ultraviolet spectrum. *p*H 1, λ_{max} 260 mμ, ϵ_{max} 16,800.

Anal. Calcd. for $C_9H_{13}N_5O_3.H_2O$: C, 42.01; H, 5.87; N, 26.22. Found: C, 42.18; H, 5.94; N, 25.78.

Water could be removed by heating the sample at 170° for 30 seconds.[16]

Calcd. for $C_9H_{13}N_5O_3$: N, 29.28. Found: N, 29.30.

8-Bis(β-hydroxyethyl)aminoguanine. This compound was obtained in 89% yield by the reaction of 8-bromoguanine with diethanolamine in methyl cellosolve and recrystallized from a large volume of water; m.p. 321° dec. Ultraviolet spectrum. *p*H 1, λ_{max} 253 mμ, ϵ_{max} 17,000.

Anal. Calcd. for $C_9H_{14}N_6O_3$: C, 42.51; H, 5.55; N, 33.05. Found: C, 42.59; H, 5.61, N, 32.96.

8-Bis(β-hydroxyethyl)aminoxanthine. This compound was obtained in 47% yield by the reaction of 8-chloroxanthine with diethanolamine in methyl cellosolve and recrystallized from water; m.p. 246° dec. Ultraviolet spectrum. *p*H 1, λ_{max} 299 mμ, ϵ_{max} 101,000.

Anal. Calcd. for $C_9H_{13}N_5O_4$: C, 42.35; H, 5.13; N, 27.44. Found: C, 42.37; H, 5.28; N, 27.32.

8-Bis(β-chloroethyl)aminoadenine hydrochloride. A mixture of 0.4 g. (0.001678 mole) of 8-bis(β-hydroxyethyl)aminoadenine, 10 ml. of thionyl chloride, and 0.5 ml. of dimethylformamide in 30 ml. of chloroform was refluxed under nitrogen for 3 hr. The solvent was removed under vacuum and the residue dissolved in absolute ethanol which was saturated with anhydrous hydrogen chloride. After cooling, 0.5 g. (85.5%) of white product was obtained and recrystallized four times from ethanol-ether, m.p., 155–162°. Ultraviolet spectrum. Methanol, λ_{max} 295 mμ, ϵ_{max} 15,900.

Anal. Calcd. for $C_9H_{14}Cl_4N_6$: C, 31.02; H, 4.05; N, 24.11; Cl, 40.73. Found: C, 30.95; H, 4.61; N, 24.19; Cl, 39.28.

8-Bis(β-chloroethyl)aminohypoxanthine hydrochloride. This material was obtained from 8-bis(β-hydroxyethyl)aminohypoxanthine and thionyl chloride in chloroform in 88% yield and recrystallized from ethanol-ether to yield white crystals m.p. 190–193° dec. Ultraviolet spectrum. Methanol, λ_{max} 271 mμ, ϵ_{max} 14,600.

Anal. Calcd. for $C_9H_{12}N_5Cl_3O \cdot {}^1/_2H_2O$: C, 33.61; H, 3.91; N, 21.77; Cl, 33.07. Found: C, 33.43; H, 4.04; N, 21.68; Cl, 33.44.

8-Bis(β-chloroethyl)aminoxanthine hydrochloride. This ma-

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terial was obtained by an analogous procedure in 19% yield and recrystallized from ethanol-ether to yield white crystals, m.p. 155° dec. Ultraviolet spectrum. Methanol, λ_{max} 296 mμ, ϵ_{max} 13,000.

Anal. Calcd. for $C_9H_{12}Cl_3N_5O_2$: C, 32.89; H, 3.68. Found: C, 33.14; H, 3.94.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE INSTITUTE OF INDUSTRIAL MEDICINE, NEW YORK UNIVERSITY MEDICAL CENTER]

Epoxidation and Cyclization of Squalene[1]

BENJAMIN L. VAN DUUREN AND F. LOUIS SCHMITT[2]

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The epoxidation of squalene, isosqualene and tetracyclosqualene was examined. It is shown that epoxidation of squalene with peracetic acid proceeds stepwise. Di-, tetra- and hexaepoxysqualenes and diepoxytetracyclosqualene were isolated and studied. Some of the lead tetraacetate cleavage products of the diols derived from these epoxides were identified.

In a recent report from this laboratory[3] the isolation and identification of squalene and isosqualene from cigarette smoke condensate was described. In view of the possibility that epoxides of squalene and isosqualene, which may also be present in cigarette smoke, may exhibit biological activity, these compounds were prepared. The rate of epoxidation of squalene and the preparation and examination of squalene epoxides are described in this report. These compounds are currently being examined for carcinogenic activity to mouse skin. In the course of this work isosqualene and tetracyclosqualene were prepared and some properties and reactions of these compounds were studied.

Raymond[4] utilized the autoxidation of benzaldehyde to epoxidize a number of unsaturated compounds including squalene. The products of the epoxidation were not isolated or identified.

Some studies on the rate of the peracetic acid oxidation of squalene were carried out in our work. Squalene was epoxidized at $-12°$ with chloroform as solvent. Two moles of peracid are consumed within fifteen minutes; another two moles of peracid are consumed only after two hours. For consumption of the last two moles of peracid it was necessary to allow reaction to proceed at 3° overnight.

Squalene was then treated with two, four, and six moles of peracetic acid in three experiments. The diepoxy product could not be purified as such. The crude product was catalytically hydrogenated and then purified by molecular distillation. The product had the correct analysis for diepoxyoctahydrosqualene. From the other two experiments there were obtained a tetra- and a hexaepoxysqualene. Tetraepoxysqualene absorbed two moles of hydrogen to give a product which had the correct analysis for tetraepoxytetrahydrosqualene. The crude epoxides showed in their infrared absorption spectra weak bands in the hydroxyl and carbonyl regions indicative of impurities. The pure distilled epoxides showed no absorption in the 3 μ and 6 μ regions but showed bands between 7.95 and 8.08 μ characteristic for 1,2-epoxides.[5] Other infrared bands which are known to be characteristic for 1,2-epoxides[6–8] *e.g.*, at 11.0, 11.7 and 12.1 μ, do not appear in the squalene epoxides.

Early attempts to purify the epoxides by chromatography on acid washed alumina or on florisil were unsuccessful. The chromatographed products were usually viscous yellow sirups which showed intense hydroxyl (3 μ) and carbonyl (5.8 μ) absorption in the infrared spectra.

Epoxidation of squalene with six moles of perbenzoic acid gave a product which from its elementary analysis was a mixture of the tetra- and hexaepoxides. The product decomposed on vacuum distillation but by molecular distillation gave a liquid tetraepoxide. The hexaepoxide could not be obtained in pure form by the perbenzoic acid oxidation. Carbonyl, hydroxyl and aromatic absorption in the infrared spectrum of the crude products suggested oxirane ring opening to hydroxybenzoate structures and possibly rearrangement reactions to keto-carbonyl–containing products. Filler and co-workers[9] studied the nature of these side reactions in the epoxidation of 1-substituted cyclohexenes.

Because of the difference in the rate of consumption of two, four, and six moles of peracid it became of interest to examine the partly epoxidized prod-

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