hot. Ten ml. of this was added to the filtrate and after cooling the yellow needles were collected and dried. The azine was recrystallized from methanol to give silky needles, m.p. 220.5–221.5°, yield 2.96 g. (65.3%).

Anal. Calcd. for $C_{19}H_{16}N_2O_2$: N, 9.21. Found: N, 9.22.

Compounds 2, 3 and 4 (Table II) were prepared in the same way.

NEWARK, DELAWARE

[CONTRIBUTION FROM NEW MEXICO HIGHLANDS UNIVERSITY AND ARIZONA STATE COLLEGE]

Potential Purine Antagonists. XI. Synthesis of Some 9-Aryl(alkyl)-2,6-disubstituted Purines

BY HENRY C. KOPPEL AND ROLAND K. ROBINS[1]

RECEIVED NOVEMBER 30, 1957

The preparation of various 9-aryl(alkyl)-2,6-dihydroxypurines (III) has been accomplished by the treatment of the corresponding 9-aryl(alkyl)-2,6-dihydroxy-8-purinethiol (II) with Raney nickel. This type of synthesis has been extended to the preparation of several 2-amino-9-alkyl-6-hydroxypurines (X) from IX. 2,6-Dihydroxy-9-phenylpurine (III, R = C_6H_5) was treated with phosphorus oxychloride and phosphorus pentachloride to give 2,6-dichloro-9-phenylpurine (IV). Several new 9-phenyl-2,6-disubstituted purines have been prepared from IV. 2-Amino-9-methyl-6-purinethiol (XI) has been prepared.

During the course of a program designed for the preparation of new biologically active purine antagonists, it appeared desirable to synthesize certain 9-aryl-2,6-disubstituted purines. A survey of the literature revealed only a patent reference[2] to the preparation of 9-phenylxanthine (9-phenyl-2,6-dihydroxypurine). Biltz and co-workers[3] successfully synthesized 2,6-dihydroxy-9-methylpurine by the treatment of uramil with methyl isothiocyanate and cyclized the substituted thiourea (I, R = CH_3) with concentrated hydrochloric acid to give 2,6-dihydroxy-9-methyl-8-purinethiol (II, R = CH_3). This compound (II, R = CH_3) was then treated with nitrous acid to give 2,6-dihydroxy-9-methylpurine (III, R = CH_3). This synthetic route succeeded for the preparation of 2,6-dihydroxy-9-ethylpurine (III, R = C_2H_5), but when 2,6-dihydroxy-9-phenyl-8-purinethiol (II, R = C_6H_5) was treated with nitrous acid by Biltz, *et al.*,[3] the expected 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5) was not obtained. Blicke and Schaaf[4] have recently utilized Raney nickel to remove the 8-mercapto group of several 9-alkyl-1,3-dimethylpurine - 2,6 - dione - 8 - thiols. Following this lead, 2,6-dihydroxy-9-phenyl-8-purinethiol (II, R = C_6H_5) was treated with Raney nickel in a sodium hydroxide solution to yield 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5) in 46% yield, or an over-all yield of 18% from uramil. In a similar manner, 9-*p*-chlorophenyl-2,6-dihydroxypurine (II, R = ClC_6H_4) was prepared from uramil in an over-all yield of 12%. This work was extended to include the preparation of -9-ethyl, -9-isobutyl and -9-methyl-2,6-dihydroxypurine by the removal of the 8-mercapto group with Raney nickel.

Since a rather large quantity of uramil was required for this study, it was found convenient to nitrosate barbituric acid after the manner of Bayer[5] and to reduce this product (violuric acid) with sodium hydrosulfite to give uramil. This procedure was found to be superior to the nitration of barbituric[6a] acid followed by the reduction of the 5-nitrobarbituric acid with stannous chloride to give uramil.[6b] Gulland[7] has reported that the chlorination of 2,6-dihydroxy-9-methylpurine with phosphorus oxychloride at 140° in a sealed tube gives 2,6 - dichloro - 9 - methylpurine. Preliminary efforts in this Laboratory to chlorinate 2,6-dihydroxy-9-methylpurine by refluxing phosphorus oxychloride with or without N,N-dimethylaniline were not successful. Treatment with phosphorus oxychloride and phosphorus pentachloride appeared to lead to degradation products. When 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5) was treated with phosphorus oxychloride for a relatively short period of time in the presence of phosphorus pentachloride, an ether-insoluble, phosphorus-containing compound was isolated from the reaction mixture. However, when the amount of phosphorus pentachloride was increased and the chlorination time extended to 40 hr., a 50% yield of 2,6-dichloro-9-phenylpurine (IV) was obtained. Treatment of IV with thiourea in refluxing alcoholic solution gave 9-phenyl-2,6-purinedithiol in good yield. When IV was treated with various amines in alcoholic solution, the corresponding 2-chloro-9-phenyl-6-substituted aminopurine (V) was obtained. Boiling dilute sodium hydroxide converted IV to 2-chloro-6-hydroxy-9-phenylpurine (VII). The assignment of structures of V and VII was made since Montgomery and Holum[8] recently have shown that with 2,6-dichloropurine the "6"-position is most readily attacked by nucleophilic reagents.

Treatment of III, R = C_6H_5, with phosphorus pentasulfide in pyridine gave 2-hydroxy-9-phenyl-6-purinethiol (VI). The assignment of structure to VI was made from the ultraviolet absorption spectrum which exhibited λ_{max} of 340 mμ at *p*H 11. The ultraviolet absorption above 300 mμ is characteristic of a 6-mercapto group.[9] This reaction is

(1) Department of Chemistry, Arizona State College, Tempe, Arizona. To whom inquiries regarding this paper should be sent.

(2) German Patent 120,437; *Chem. Zentr.*, **72**, I, 1219 (1901).

(3) H. Biltz, K. Strufe, E. Topp, M. Heyn and R. Rohl, *Ann.*, **423**, 200 (1921).

(4) F. F. Blicke and R. L. Schaaf, THIS JOURNAL, **72**, 5857 (1956).

(5) A. von Bayer, *Ann.*, **127**, 210 (1920).

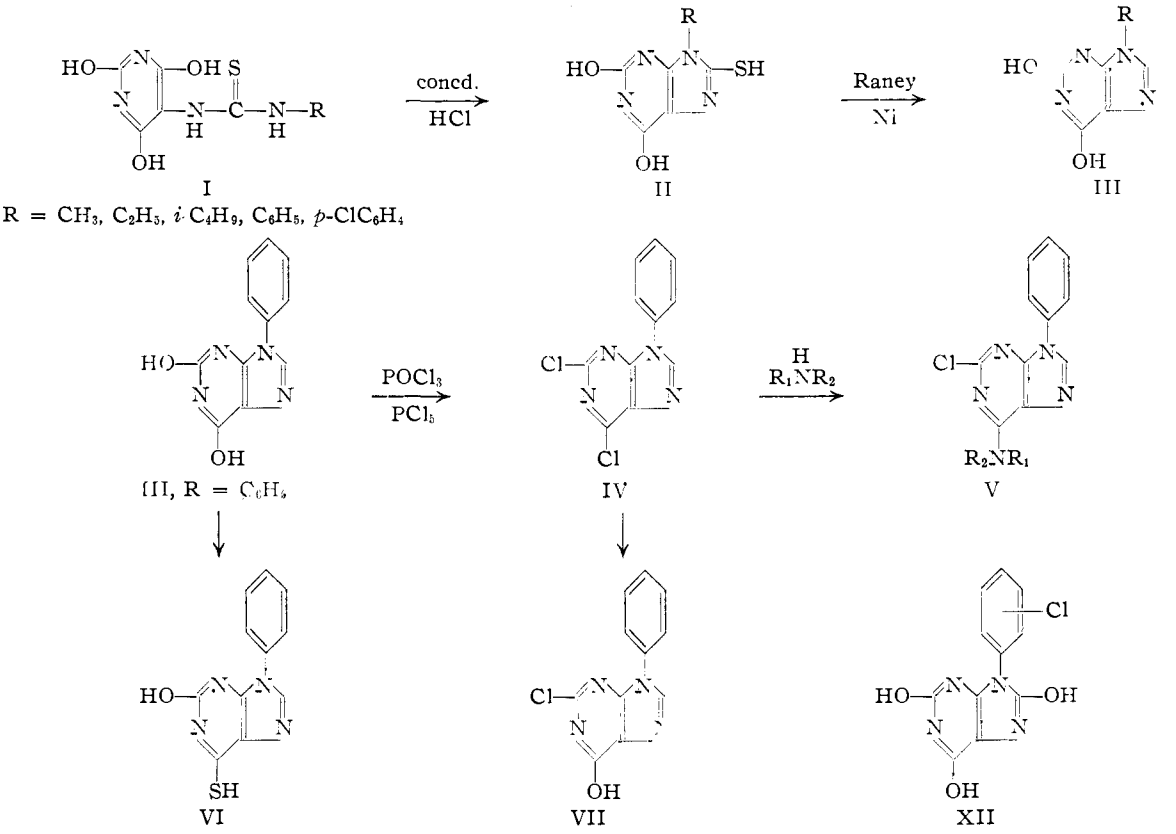
(6) (a) W. W. Hartman and O. E. Shephard, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 440; (b) p. 617.

(7) J. M. Gulland, *J. Chem. Soc.*, 647 (1938).

(8) J. A. Montgomery and L. B. Holum, THIS JOURNAL, **79**, 2185 (1957).

(9) A. G. Beaman, *ibid.*, **76**, 5633 (1954).

REACTION SCHEME I

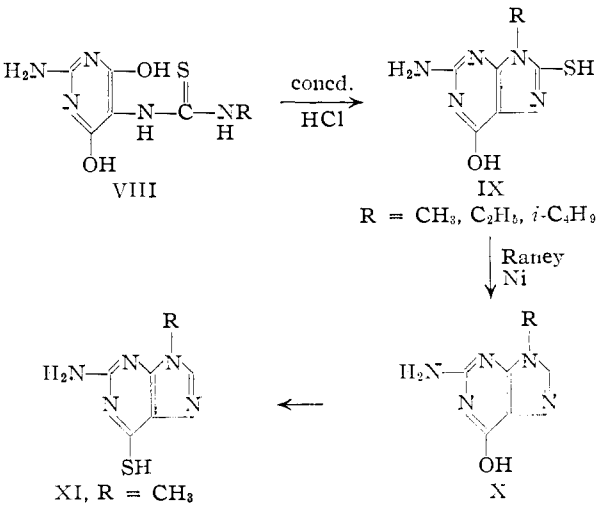


not unexpected since Beaman[9] found that xanthine under similar conditions gave 2-hydroxy-6-purinethiol.

The general method of synthesis of 9-aryl(alkyl)-2,6-dihydroxypurines was extended to the preparation of several 9-alky-2-amino-6-hydroxypurines as indicated in reaction scheme II. The starting material in this instance was 2,5-diamino-4,6-dihydroxypyrimidine which was prepared by the nitrosation of 2-amino-4,6-dihydroxypyrimidine[10] followed by reduction of the 5-nitroso derivative with sodium hydrosulfite. When 2,5-diamino-4,6-dihydroxypyrimidine was treated with an alkyl or aryl isothiocyanate, VIII was prepared in good yield. When R was alkyl, VIII was converted smoothly to the corresponding 9-alkyl-2-amino-6-hydroxy-8-purinethiol (IX) with boiling hydrochloric acid. Treatment of the 9-alkyl-2-amino-6-hydroxy-8-purinethiol (IX) with Raney nickel in a basic solution gave the corresponding 9-alkyl-2-amino-6-hydroxypurine (X). In this manner 9-methyl, 9-ethyl, and 9-isobutyl-2-amino-6-hydroxypurine were successfully prepared in an over-all yield of 19, 18 and 16%, respectively, from 2,5-diamino-4,6-dihydroxypyrimidine. Gulland and Story[11] have previously reported the synthesis of 2-amino-6-hydroxy-9-methylpurine (9-methylguanine) in two steps from 2,6-dichloro-9-methylpurine.

The ultraviolet absorption spectra for X, R = CH_3, as reported by Gulland and Story,[11] and that exhibited by 2-amino-6-hydroxy-9-methylpurine prepared in this Laboratory showed good agreement. When X, R = CH_3, was treated with phosphorus pentasulfide in pyridine solution, 2-amino-9-methyl-6-purinethiol (XI) was obtained. This latter compound is of interest since 2-amino-6-purinethiol has exhibited interesting anti-tumor properties.[12]

REACTION SCHEME II



When "R" (Reaction Scheme II) was aryl, the intermediate, thioureide VIII, could not be successfully cyclized to give IX. Even when dilute hy-

(10) E. Buttner, *Ber.*, **36**, 2229 (1903).

(11) J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 692 (1938).

(12) F. S. Philips, S. S. Sternberg, L. D. Hamilton and D. A. Clarke, *Proc. Am. Assoc. Cancer Research*, **1**, 37 (1954); J. H. Burchenal, *Federation Proc.*, **13**, 700 (1954).

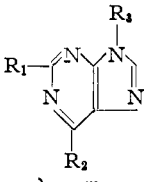
drochloric acid was employed, only degradation products were obtained instead of the purine IX, R = C_6H_5.

Two new 9-aryl-2,6,8-trihydroxypurines (XII) were prepared by the general method of Fischer[13] for the preparation of 9-phenyluric acid. The yield of 9-*p*-chlorophenyl-2,6,8-trihydroxypurine (XIV) from uramil was 50%. Similarly, 9-*o*-chlorophenyl-2,6,8-trihydroxypurine was obtained in a 50% over-all yield.

The ultraviolet absorption spectra of the 9-aryl-(alkyl)-2,6-disubstituted purines prepared are recorded in Table I.

TABLE I

THE ULTRAVIOLET ABSORPTION SPECTRA OF SOME 9-ARYL(ALKYL)-2,6-DISUBSTITUTED PURINES



R_1	R_2	R_3	λ_{max}, mμ *p*H 1	ε	λ_{max}, mμ *p*H 11	ε
OH	OH	C_6H_5	260	13,110	275	12,500
OH	OH	*p*-ClC_6H_4	260	22,350	275	14,500
			225	13,100		
OH	SH	C_6H_5	337	30,700	340	26,600
NH_2	OH	CH_3	252	14,200	267	12,400
			280	10,000		
OH	OH	CH_3	235	11,100	247	14,500
			262	14,900	277	14,500
SH	SH	C_6H_5	330	27,300	344	28,600
Cl	Cl	C_6H_5	275[a]	25,200[a]		
Cl	OH	C_6H_5	260	14,800	252	12,400
OH	OH	C_2H_5	235	6,700	247	9,000
			262	9,000	278	9,000
OH	OH	*i*-C_4H_9	235	9,300	248	8,900
			262	10,400	280	8,900
NH_2	OH	C_2H_5	252	12,400	270	10,800
			280	8,100		
NH_2	OH	*i*-C_4H_9	252	11,600	270	10,400
			280	7,900		
NH_2	SH	CH_3	227	10,100	260	6,000
			250	7,200	350	13,200
			320	11,500		

[a] Absorption spectrum determined in absolute ethanol.

Experimental Procedure[14]

Preparation of 5-Amino-2,4,6-trihydroxypyrimidine (Uramil[6b]).—One hundred grams of barbituric acid was added to 1 liter of water, and the mixture was heated to 70–80°, at which time 40 g. of sodium nitrite was added. After the solution had been allowed to stand for 10 min., 200 g. of sodium hydrosulfite was added a little at a time with continuous stirring. The temperature was kept below 90° during the addition, and finally cooled to room temperature. The 5 aminobarbituric acid was filtered, washed with a little cold water, and dried in a vacuum desiccator. This product, 92 g., was used directly without further purification.

Anal. Calcd. for $C_4H_5N_3O_3$: N, 29.5. Found: N, 29.8.

N-(2,4,6-Trihydroxy-5-pyrimidyl)-N'-phenylthiourea (I, R = C_6H_5) was synthesized using a modification of the method of Biltz, *et al.*[3] Seventy grams of uramil was dissolved in 1500 ml. of *N* sodium hydroxide and the solution heated to 60°; 66 g. of phenyl isothiocyanate was added dropwise to the solution with constant stirring. The addition of the isothiocyanate took approximately 1.5 hr., and during this time the color changed from deep violet to a light yellow. After all the isothiocyanate had been added, the solution was stirred at 60° for an additional 2 hr. The solution was acidified with glacial acetic acid, cooled, and filtered and the product washed with cold water. The yield was 95 g. Recrystallization from dilute glacial acetic acid gave colorless plates, m.p. >300°.

Anal. Calcd. for $C_{11}H_{11}N_4O_3S$: N, 20.1. Found: N, 20.6.

Preparation of 2,6-Dihydroxy-9-phenylpurine (III, R = C_6H_5).—Fifty grams of 2,6-dihydroxy-9-phenyl-8-purinethiol (II, R = C_6H_5)[3] was dissolved in 500 ml. of *N* sodium hydroxide; 150 g. of wet Raney nickel was added to the solution, and the mixture was refluxed for 3 hr. The Raney nickel was then filtered and the filtrate cooled to 4°. The sodium salt of 2,6-dihydroxy-9-phenylpurine crystallized and was filtered. A second 150 g. of wet Raney nickel was added to the filtrate and this mixture refluxed for another 3 hr. At the end of this time the sodium salt of the 2,6-dihydroxy-9-phenylpurine was isolated as before. The combined sodium salts were dissolved in boiling water and the solution treated with charcoal. The hot filtrate was acidified with concentrated hydrochloric acid to yield white, shiny plates of 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5), m.p. >300°. Yield of product was 20 g.

Anal. Calcd. for $C_{11}H_7N_4O_2$: C, 57.8; H, 3.5; N, 24.6. Found: C, 57.5; H, 3.4; N, 24.6.

Preparation of 2-Hydroxy-9-phenyl-6-purinethiol (VI).—Eight grams of 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5) plus 24 g. of phosphorus pentasulfide were ground together in a mortar and then transferred to a flask containing 500 ml. of anhydrous pyridine. The solution was refluxed for 3 hr. The excess pyridine was distilled under reduced pressure, and 500 ml. of ice-water was added to the residue. The solution was allowed to stand at room temperature and finally refluxed for 2 hr. The aqueous solution was acidified with hydrochloric acid and cooled. The yield of product was 4.5 g. For purification the compound was reprecipitated twice from hot, dilute potassium hydroxide solution and finally recrystallized from 40% glacial acetic acid to yield long, light-yellow needles, m.p. >300°. This product analyzed for a monohydrate which lost a mole of water when heated at 180°.

Anal. Calcd. for $C_{11}H_8N_4OS \cdot H_2O$: C, 50.4; H, 4.1; N, 21.4. Found: C, 50.5; H, 4.1; N, 21.3. Calcd. for $C_{11}H_8N_4OS$: C, 54.1; H, 3.3. Found: C, 53.9; H, 3.2.

Preparation of 2,6-Dichloro-9-phenylpurine (IV).—Twenty grams of 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5) was added to 500 ml. of phosphorus oxychloride and 100 g. of phosphorus pentachloride and the mixture refluxed for 40 hr. Solution occurred after one hour. The excess phosphorus oxychloride was distilled under reduced pressure and the residue poured on cracked ice with very vigorous stirring. The solution was extracted six times with a liter of ether each time. The precipitate which had not dissolved in the ether was filtered and saved. The ether was distilled to yield 12 g. of light-yellow, amorphous crystals, m.p. 240–242°. Recrystallization from ethyl acetate gave pale-yellow needles, which sublimed at 193° and melted at 244–246°.

Anal. Calcd. for $C_{11}H_6N_4Cl_2$: C, 49.8; H, 2.3; N, 21.2. Found: C, 49.5; H, 2.4; N, 20.9.

The ether-insoluble product which weighed 3 g. was boiled in *N* sodium hydroxide to yield 1.2 g. of 2-chloro-6-hydroxy-9-phenylpurine (VII).

Preparation of 2-Chloro-6-hydroxy-9-phenylpurine (VII).—Three grams of 2,6-dichloro-9-phenylpurine (IV) was refluxed in *N* sodium hydroxide for 3 hr. At the end of this period the solution was treated with charcoal and the filtrate chilled. The sodium salt of 2-chloro-6-hydroxy-9-phenylpurine crystallized. The product was filtered, washed with a little ice-water, and dissolved in boiling water. The solution was acidified with glacial acetic acid while hot to yield light-yellow needles, m.p. 276–278°. The yield of product was 1.9 g. Recrystallization from ethanol gave long, white needles, m.p. 280–281°.

Anal. Calcd. for $C_{11}H_7N_4OCl$: C, 53.4; H, 2.8. Found: C, 53.1; H, 2.9.

(13) E. Fischer, *Ber.*, **33**, 1701 (1900).

(14) All melting points were taken on the Fisher–Johns melting point apparatus unless otherwise stated.

Preparation of 9-Phenyl-2,6-purinedithiol.—To 200 ml. of absolute methanol containing 10 g. of thiourea was added 5 g. of 2,6-dichloro-9-phenylpurine (IV). The mixture was refluxed on the steam-bath for 6 hr. Complete solution took place after 0.5 hr. and the product crystallized from the hot reaction mixture after 1 hr. After 6 hr. the mixture was chilled to yield 3 g. of yellow-green needles which were filtered and washed with a little cold methanol. The product was recrystallized from 90% ethanol to yield light-green needles, m.p. >300°.

Preparation of 2-Chloro-6-*n*-propylamino-9-phenylpurine (V, R_1 = H, R_2 = *n*-C_3H_7).—To 100 ml. of ethanol containing 5 g. of 2,6-dichloro-9-phenylpurine (IV) was added 12 ml. of *n*-propylamine. The mixture was heated on the steam-bath, and solution occurred almost immediately. It was then heated for an additional 3 hr. The cooled solution yielded 4.0 g. of white product, m.p. 118–120°. Recrystallization from 80% ethanol gave white needles, m.p. 121–122°.

Anal. Calcd. for $C_{14}H_{14}N_5Cl$: C, 58.2; H, 4.8. Found: C, 57.8; H, 4.7.

Preparation of 2-Chloro-6-β-phenylethylamino-9-phenylpurine Hydrochloride (V, R_1 = H, R_2 = $C_6H_5CH_2CH_2$).—To 75 ml. of absolute ethanol containing 1.9 g. of β-phenylethylamine was added 4 g. of 2,6-dichloro-9-phenylpurine (IV). The solution was heated on the steam-bath for 1.5 hr., treated with charcoal, and filtered. Dry HCl gas was passed into the cooled filtrate for 20 min. At the end of this time the solution yielded 5.4 g. of white crystals, m.p. 168–170°. Recrystallization from absolute ethanol raised the m.p. to 172–174°.

Anal. Calcd. for $C_{19}H_{17}N_5Cl\cdot HCl$: C, 58.9; H, 4.4. Found: C, 58.5; H, 4.4.

Preparation of 2-Chloro-6-N,N-dimethylamino-9-phenylpurine (V, R_1, R_2 = CH_3).—To 70 ml. of water containing 5 g. of 2,6-dichloro-9-phenylpurine (IV) was added 20 ml. of 40% aqueous dimethylamine. The mixture was heated on the steam-bath for 8 hr., cooled, and the crude product filtered and washed with a little cold ethanol. The yield was 3.5 g. The crude product was crystallized once from ethanol to yield white crystals, m.p. 166–168°. For analysis the product was recrystallized again from ethanol to yield white needles, m.p. 168–169°.

Anal. Calcd. for $C_{13}H_{12}N_6Cl$: C, 57.2; H, 4.4. Found: C, 57.2; H, 4.4.

Preparation of 2,6-Dihydroxy-9-*p*-chlorophenyl-8-purinethiol (II, R = *p*-ClC_6H_4).—Forty grams of I, R = *p*-C_6H_4Cl, prepared in a manner similar to the preparation of I, R = C_6H_5, was refluxed in 650 ml. of concentrated hydrochloric acid for 5 hr. No true solution occurred, and at the end of the 5 hr. the mixture was diluted to 1 liter with water and the product filtered immediately and washed with water to yield 23 g. Recrystallization from dilute acetic acid gave light-yellow crystals, m.p. >300°.

Anal. Calcd. for $C_{11}H_7N_4O_2SCl$: N, 19.0. Found: N, 19.0.

Preparation of 2,6-Dihydroxy-9-*p*-chlorophenylpurine (III, R = *p*-ClC_6H_4).—Thirty grams of II, R = *p*-ClC_6H_4, was dissolved in 500 ml. of *N* sodium hydroxide; 90 g. of wet Raney nickel was added to the solution and the mixture refluxed for 3 hr. At the end of this time the Raney nickel was filtered and the solution cooled to 10° to give the sodium salt of 2,6-dihydroxy-9-*p*-chlorophenylpurine. The product was isolated and purified as for 2,6-dihydroxy-9-phenylpurine (III, R = C_6H_5). The yield was 9.0 g. An analytical sample was obtained by recrystallization from dilute acetic acid. The product was dried at 180° for 24 hr. for analysis.

Anal. Calcd. for $C_{11}H_7N_4O_2Cl$: C, 50.5; H, 2.6; N, 21.3. Found: C, 50.8; H, 2.5; N, 21.0.

Preparation of 2,6-Dihydroxy-9-methylpurine (III, R = CH_3).—Ten grams of II, R = CH_3, prepared by the method of Biltz[3] was treated with Raney nickel as for the preparation of III, R = C_6H_5. The product was isolated as the sodium salt and finally converted with glacial acetic acid to white crystals of 2,6-dihydroxy-9-methylpurine (III, R = CH_3). The yield of product was 4.8 g. For analysis the product was recrystallized from dilute acetic acid to yield white crystals, m.p. >300°.

Anal. Calcd. for $C_6H_6N_4O_2$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.8; H, 3.7; N, 33.8.

Similarly, 17.0 g. of 2,6-dihydroxy-9-ethyl-8-purinethiol[2] gave 6 g. of 2,6-dihydroxy-9-ethylpurine (III, R = C_2H_5) which was purified for analysis from dilute acetic acid.

Anal. Calcd. for $C_7H_8N_4O_2$: C, 46.8; H, 4.4; N, 31.1. Found: C, 47.2; H, 4.7; N, 30.6.

Preparation of 2,6-Dihydroxy-9-isobutyl-8-purinethiol (II, R = *i*-C_4H_9).—Twenty-five grams of N-(2,4,6-trihydroxy-5-pyrimidyl)-N′-isobutylthiourea, prepared in analogous fashion to N-(2,4,6-trihydroxy-5-pyrimidyl)-N′-phenylthiourea using isobutyl isothiocyanate instead of phenyl isothiocyanate, was refluxed in 250 ml. of concentrated hydrochloric acid for 5 hr. No true solution occurred, and at the end of this time the mixture was diluted to 500 ml. with water. The light-yellow crystals were filtered immediately and washed with a little water; yield of product 16 g. For analysis the product was reprecipitated twice from dilute sodium hydroxide with acetic acid.

Anal. Calcd. for $C_9H_{12}N_4O_2S$: C, 45.0; H, 5.0. Found: C, 45.0; H, 4.8.

Preparation of 2,6-Dihydroxy-9-isobutylpurine (III, R = *i*-C_4H_9).—Ten grams of 2,6-dihydroxy-9-isobutyl-8-purinethiol was dissolved in 200 ml. of *N* sodium hydroxide; 30 g. of wet Raney nickel was added to the solution and the mixture refluxed for 3 hr. The product was purified *via* the solution salt to yield 5.0 g.

Anal. Calcd. for $C_9H_{12}N_4O_2$: C, 51.9; H, 5.7. Found: C, 51.6; H, 5.8.

Preparation of 2,5-Diamino-4,6-dihydroxypyrimidine (IX).—One hundred grams of 2-amino-4,6-dihydroxypyrimidine[10] was dissolved in 800 ml. of 0.5 *N* sodium hydroxide. The solution was heated to 60°, and 40 g. of sodium nitrite was added. Concentrated hydrochloric acid was added carefully to the solution until a pink precipitate was observed, which was filtered and washed with a little water. The crude 2-amino-4,6-dihydroxy-5-nitrosopyrimidine was suspended in 1 liter of water at 20°, and 25 g. of sodium hydrosulfite was added carefully. The mixture was boiled gently for 5 min. and then filtered hot; yield of product 38 g. For analysis the compound was recrystallized from water.

Anal. Calcd. for $C_4H_6N_4O_2$: C, 33.8; H, 4.2; N, 39.4. Found: C, 33.5; H, 3.9; N, 39.0.

Preparation of 2-Amino-6-hydroxy-9-methylpurine (XI, R = CH_3).—Twenty-five grams of 2,5-diamino-4,6-dihydroxypyrimidine was dissolved in 400 ml. of *N* sodium hydroxide and the solution heated to 60–70°. Thirteen grams of methyl isothiocyanate was added to the solution in one portion and the solution stirred for 4 hr. The solution was then acidified with glacial acetic acid and allowed to stand at room temperature for 6 hr. The light-yellow product was filtered and washed with a little cold water. The yield of N-(2-amino-4,6-dihydroxy-5-pyrimidyl)-N′-methylurea was 25 g. Several attempts made to purify this intermediate were unsuccessful. The compound became highly colored upon prolonged exposure to the air. This crude product was refluxed in 250 ml. of concentrated hydrochloric acid for 5 hr. At the end of 5 hr. the mixture was diluted to 450 ml. with water. The light-yellow crystals were filtered immediately and washed with a little water. The yield of 2-amino-6-hydroxy-9-methyl-8-purinethiol was 14 g. This crude product was dissolved in 250 ml. of *N* sodium hydroxide; 42 g. of wet Raney nickel was added to the solution, and the mixture was refluxed for 3 hr. At the end of this time, the Raney nickel was filtered and the solution cooled. To the cooled filtrate was added 42 g. of wet Raney nickel, and the mixture was refluxed for an additional 3 hr. At the end of this time the Raney nickel was filtered and the filtrate treated with charcoal and acidified with glacial acetic acid. The yield of product was 7.5 g., m.p. >300°. For analysis the product was recrystallized from dilute N,N-dimethylformamide.

Anal. Calcd. for $C_6H_7N_5O$: C, 43.7; H, 4.2; N, 42.4. Found: C, 43.9; H, 4.6; N, 41.8.

Preparation of 2-Amino-6-hydroxy-9-isobutylpurine (XI, R = *i*-C_4H_9).—Twenty-three grams of 2,5-diamino-4,6-dihydroxypyrimidine (IX) was treated with 20 g. of isobutyl isothiocyanate and the resulting N-(2-amino-4,6-dihydroxy-5-pyrimidyl)-N′-isobutylthiourea isolated and cyclized in hydrochloric acid as for the preparation of 2-amino-6-hydroxy-9-methyl-8-purinethiol. The crude 2-amino-6-hydroxy-9-isobutyl-8-purinethiol, 12 g., was dissolved in

250 ml. of *N* sodium hydroxide and the solution refluxed with Raney nickel (40 g.) for 3 hr. The solution was filtered and an additional 40 g. of wet Raney nickel added and the solution refluxed for an additional 3 hr. The solution was cooled and filtered and the filtrate acidified with acetic acid to yield 5.1 g. of 2-amino-6-hydroxy-9-isobutylpurine (XI, R = *i*-C_4H_9). The product was dissolved in dilute hydrochloric acid and the hot solution neutralized with ammonium hydroxide. The white product was filtered and washed with water and a small amount recrystallized from N,N-dimethylformamide for analysis.

Anal. Calcd. for $C_9H_{13}N_5O$: C, 52.5; H, 6.3. Found: C, 52.2; H, 6.7.

Preparation of 2-Amino-9-ethyl-6-hydroxypurine (XI, R = C_2H_5).—Twenty-five grams of 2,5-diamino-4,6-dihydroxypyrimidine (IX) was treated with 17 g. of ethyl isothiocyanate. The resulting product was isolated and cyclized with hydrochloric acid to give 14 g. of 2-amino-6-hydroxy-9-ethyl-8-purinethiol (XI, R = C_2H_5). The procedure followed in this preparation and in converting XI, R = C_2H_5, to 2-amino-9-ethyl-6-hydroxypurine with Raney nickel was essentially the same as for the preparation of 2-amino-9-methyl-6-hydroxypurine (XII, R = CH_3). The yield of 2-amino-9-ethyl-6-hydroxypurine (XI, R = C_2H_5) thus obtained was 6.0 g.

Anal. Calcd. for $C_7H_9N_5O$: C, 46.9; H, 5.0. Found: C, 46.7; H, 5.4.

Preparation of 2-Amino-9-methyl-6-purinethiol (XIII, R = CH_3).—Eight grams of 2-amino-6-hydroxy-9-methylpurine (XI) and 32 g. of phosphorus pentasulfide were ground together in a mortar and then transferred to a flask containing 500 ml. of dry pyridine. The mixture was refluxed for 8 hr. The excess pyridine was distilled under reduced pressure and 500 ml. of ice-water carefully added to the residue in the flask. The solution was then heated 3 hr. on the steam-bath and finally chilled overnight. The yield of crude product was 5.0 g. For purification the compound was reprecipitated twice from hot, dilute sodium hydroxide with acetic acid and finally recrystallized from dilute acetic acid to yield light-yellow crystals, m.p. >300°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.8; H, 3.9. Found: C, 39.9; H, 3.9.

Preparation of 9-*p*-Chlorophenyluric Acid (XII).—Seventy-one grams of uramil was dissolved in 1.5 l. of *N* sodium hydroxide and the solution heated to 60–70°; 75 g. of *p*-chlorophenyl isocyanate was added dropwise to the solution with constant stirring. The addition of the isocyanate required approximately 1.5 hr. After all the isocyanate had been added, the solution was stirred at the same temperature for an additional 2 hr. The solution was cooled and acidified with glacial acetic acid, and the pale-yellow product was filtered and washed with a little cold water. The crude product was then added to 1 liter of concentrated hydrochloric acid and the solution refluxed for a period of 6 hr. The mixture was then diluted with water to 1500 ml., and the white crystals of 9-*p*-chlorophenyluric acid were filtered immediately and washed with a little water. The yield of product was 70 g. For analysis the product was recrystallized from acetic acid to give white needles, m.p. >300°. The crystals were dried at 180° for 24 hr. for analysis.

Anal. Calcd. for $C_{11}H_7N_4O_3Cl$: C, 47.4; H, 2.51; N, 20.1. Found: C, 47.2; H, 3.0; N, 19.9.

Similarly, 54 g. of uramil and 50 g. of *o*-chlorophenyl isocyanate gave 49.0 g. of 9-*o*-chlorophenyluric acid. For analysis the product was recrystallized from a dilute acetic acid solution to give white needles, m.p. >300°.

Anal. Calcd. for $C_{11}H_7N_4O_3Cl \cdot H_2O$: C, 44.5; H, 3.04; N, 18.9. Found: C, 44.8; H, 3.34; N, 18.7.

TEMPE, ARIZONA

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Purine N-Oxides. I. Mono-oxides of Aminopurines[1]

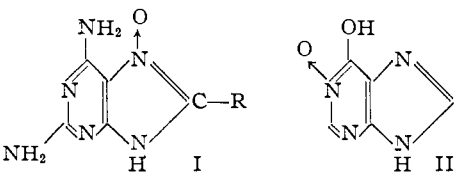
BY MARCUS A. STEVENS,[2] DAVID I. MAGRATH,[3] HERMAN W. SMITH AND GEORGE BOSWORTH BROWN

RECEIVED JANUARY 2, 1958

Mono-N-oxides have been isolated from the mixtures resulting from the oxidation of adenine (6-aminopurine), adenosine, 2',3'-isopropylideneadenosine or 2,6-diaminopurine with hydrogen peroxide–acetic acid.

Direct oxidation of purines to N-oxides has not previously been reported, although numerous examples of the oxidation of pyridines[4] and a few examples of the oxidation of pyrimidines[5–7] to N-oxides are known. The interesting influences of the N-oxide grouping on the chemical behavior of the total molecule, the possibility that such derivatives might be significant in the metabolic roles of purines in those co-enzymes which function in oxidation–reduction systems, and their possible significance in the enzymatic hydroxylation of purines *in vivo*, make purine N-oxides of interest. The possibility that N-oxides of natural purine derivatives might serve as antimetabolites is also to be considered.

Recently Timmis[8] reported that a purine 7-N-oxide (I) could be made by treating an aromatic aldehyde anil with 2,4,6-triamino-5-nitrosopyrimi-



dine, and Taylor[9] reported the synthesis of hypoxanthine 1-N-oxide (II) by orthoformate ring closure of 4-aminoimidazole-5-hydroxamic acid or of aminomalonamidine-hydroxamic acid. At the same time[10] an initial report was made that an oxide of adenine could be obtained by direct oxidation with hydrogen peroxide in glacial acetic acid.

(1) This investigation was supported in part by funds from the American Cancer Society (Grant #MET-27), National Cancer Institute, National Institutes of Health, Public Health Service (Grant #CY-3190), and from the Atomic Energy Commission (Contract #AT(30-1)-910).

(2) Fellow of Sloan-Kettering Institute.

(3) Fellow of Sloan-Kettering Institute.

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