[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY]

Potential Purine Antagonists. XIX. Synthesis of Some 9-Alkyl(aryl)-2-amino-6-substituted Purines and Related v-Triazolo[d]pyrimidines[1]

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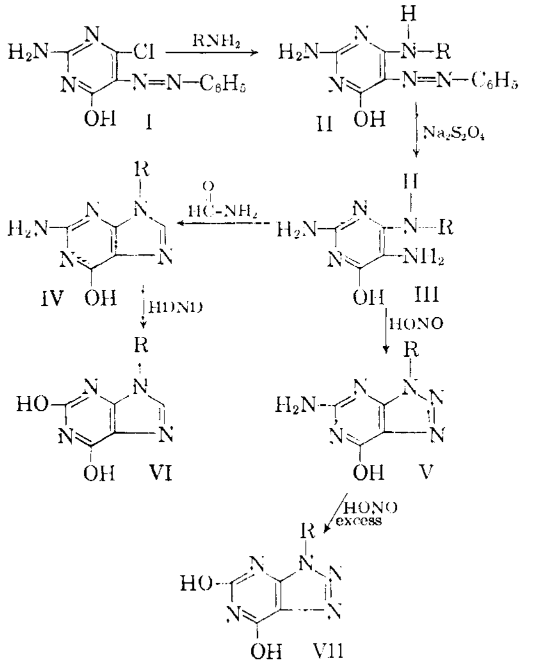
A number of new 9-alkyl(aryl)-2-amino-6-hydroxypurines (IV) have been prepared in several steps beginning with 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine (I). By ring closure of the intermediate, 4-alkyl(aryl)amino-2,5-diaminopyrimidine (III), with nitrous acid, the corresponding 3-alkyl(aryl)-5-amino-7-hydroxy-v-triazolo(d)pyrimidines (V) were formed. Some 9-aryl-2,6-diaminopurines (XIII) were prepared in several steps from 4-chloro-2,6-diaminopyrimidine (IX).

The anti-tumor activity of 5-amino-7-hydroxy-v-triazolo[d]pyrimidine[2-4] and the observation that this compound is incorporated into the nucleic acid of certain tumors[5] prompted us to investigate the synthesis of certain 5-amino-7-hydroxy-v-triazolo[d]pyrimidines possessing an alkyl or aryl substituent in position 3. Thus, these compounds represent nucleoside models of 5-amino-7-hydroxy-v-triazolo[d]pyrimidine and could conceivably



REACTION SCHEME I

possess increased anti-tumor activity over that of the parent compound. The 3-substituted-5-amino-7-hydroxy-v-triazolo[d]pyrimidines listed in Table I were prepared according to Reaction Scheme I beginning with 2-amino-6-chloro-4-hydroxy-5-phenylazopyrimidine (I) which was prepared by the method of Boon and Leigh[6] from 2-amino-4-chloro-6-hydroxypyrimidine[7] and diazotized aniline. The reaction of I with various primary amines in alcoholic solution resulted in the preparation of the corresponding 6-alkyl(aryl)amino-2-amino-4-hydroxy-5-phenylazopyrimidines (II) which were in turn reduced with sodium hydrosulfite to the corresponding 5-amino derivatives (III). Various attempts to purify the 6-alkyl(aryl)-amino-2,5-diamino-4-hydroxypyrimidines were unsuccessful. These compounds were quite unstable and decomposed rapidly on exposure to air or during attempted recrystallization. Fidler and Wood have previously commented on the instability of 2,5-diamino-4-hydroxy-6-methylaminopyrimidine.[8] These intermediates (III) were therefore converted directly with nitrous acid to the desired 3-alkyl(aryl)-5-amino-7-hydroxy-v-triazolo[d]pyrimidines listed in Table I. The 3-benzyl- and 3-cyclohexyl-5-amino-7-hydroxy-v-triazolo[d]pyrimidines (V, R = $CH_2C_6H_5$ and C_6H_{11}) were converted to the corresponding 5,7-dihydroxy-v-triazolo[d]pyrimidines (VII, R = $CH_2C_6H_5$ and C_6H_{11}) with hydrochloric acid and sodium nitrite at 90°.

Cyclization of the intermediate, 6-alkyl(aryl)-amino-2,5-diamino-4-hydroxypyrimidines, with formamide[9] provided the 9-alkyl(aryl)-2-amino-6-hydroxypurines (IV) listed in Table II. When 2-amino-6-hydroxy-9-phenylpurine (IV, R = C_6H_5) was treated with hot nitrous acid in the presence of hydrochloric acid, deamination occurred to give 2,6-dihydroxy-9-phenylpurine (VI, R = C_6H_5). The ultraviolet absorption spectra of VI, R = C_6H_5, were identical to those exhibited by a sample of the same compound prepared previously by another route.[10] It is interesting to note that a previous synthetic route[10] to 9-alkyl-2-amino-6-hydroxypurine failed to yield 2-amino-6-hydroxy-9-phenylpurine (IV, R = C_6H_5). The present method however is applicable to the preparation of both 9-alkyl- and 9-arylguanines and in general gives a better over-all yield. 2-Amino-6-hydroxy-9-isobutylpurine (IV, R = i-C_4H_9) was prepared in a 34% over-all yield from 2-amino-4-chloro-6-hydroxypyrimidine (I) and found to be identical to the same compound previously prepared[10] from 2,5-diamino-4,6-dihydroxypyrimidine.

The anti-tumor activity of 2-amino-6-purinethiol[11] prompted us to prepare several 9-alkyl(aryl)-2-amino-6-purinethiols (VIII) (see Table III) by

(1) Supported in part by research grant CY-4008 from the National Cancer Institute of the National Institutes of Health, Public Health Service and in part by a research grant from Parke, Davis & Co., Detroit 32, Mich.

(2) L. W. Law, *Cancer Research*, **10**, 186 (1950).

(3) G. W. Kidder, V. C. Dewey, R. E. Parks, Jr., and G. L. Woodside, *Science*, **109**, 511 (1949).

(4) A. Gellhorn, *Cancer*, **6**, 1030 (1953).

(5) H. George Mandel, P. Carlo and P. K. Smith, *J. Biol. Chem.*, **206**, 181 (1954).

(6) W. R. Boon and T. Leigh, *J. Chem. Soc.*, 1497 (1951).

(7) H. S. Forrest, R. Hull, H. J. Rodda and A. R. Todd, *ibid.*, 3 (1951).

(8) W. E. Fidler and H. C. S. Wood, *ibid.*, 4160 (1957).

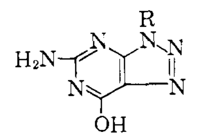
(9) R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, THIS JOURNAL, **75**, 263 (1953).

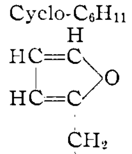
(10) H. C. Koppel and R. K. Robins, *ibid.*, **80**, 2751 (1958).

(11) D. A. Clark, G. B. Elion, G. H. Hitchings and C. C. Stock, *Cancer Research*, **18**, 445 (1958).

TABLE I

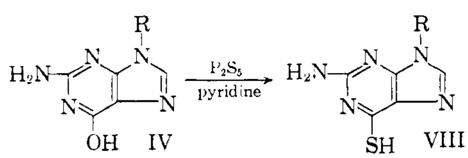
3-ALKYL(ARYL)-5-AMINO-7-HYDROXY-V-TRIAZOLO[d]PYRIMIDINES



R[a]	Over-all yield from 1, %	M.p.,[b] °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	λmax, mμ pH 1	ε	λmax, mμ pH 11	ε
o-$ClC_6H_4CH_2$	38	315–316	47.7	47.6	3.2	3.1	30.4	30.4	255	14,000	280	12,700
3,4-$Cl_2C_6H_3CH_2$	38	322–323	42.2	42.1	2.6	2.7	27.0	27.1	255	13,500	280	12,200
n-$CH_3(CH_2)_9$	33	258–259	57.5	57.2	8.2	8.3	28.8	28.8	254	14,400	279	12,800
n-$CH_3(CH_2)_7$	24	263–264	54.6	54.5	7.6	7.8	31.8	31.8	255	12,900	280	11,100
p-$ClC_6H_4CH_2$	25	327–328	47.7	47.9	3.2	3.5	30.4	30.3	255	14,100	280	14,400
$C_6H_5CH_2$	29	313–314	64.5	64.3	4.1	4.1	34.7	34.6	255	13,300	280	12,600
Cyclo-C_6H_{11}	34	311–312	51.3	51.5	6.2	5.9	36.0	36.1	255	13,100	280	12,800
	34[c]	285 d.	46.5	46.8	3.4	3.4	36.2	36.2	255	13,700	280	12,800
C_6H_5	32	326–327 d.	52.9	53.1	3.8	3.6	37.3	37.6	270	13,200	287	13,900
p-ClC_6H_4	26	>360	45.8	46.0	2.9	2.6	32.3	32.5	272	7,900	287	13,700
p-BrC_6H_4	29	>360	39.1	39.8	2.3	2.8	27.5	28.2	..	....	287	16,500
3,4-$Cl_2C_6H_3$	23	d. >300	40.5	40.7	2.0	2.0	28.5	28.5	272	10,900	289	15,200

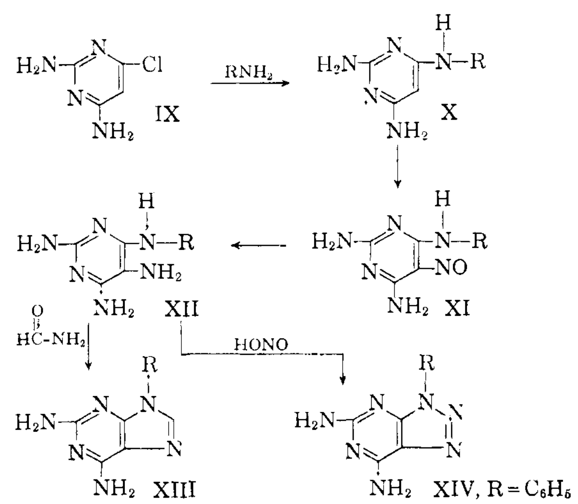
[a] Compounds recrystallized from N,N-dimethylformamide. [b] Melting points >300° determined on copper block and are uncorrected. [c] Recrystallized from dilute acetic acid.

treatment of IV with phosphorus pentasulfide in pyridine, similar to the procedure previously employed by Elion and Hitchings for the preparation of 2-amino-6-purinethiol from guanine.[12]



IV VIII

The anti-tumor activity exhibited by 2,6-diaminopurine[13] which has been demonstrated to act as a precursor of nucleic acid guanine in the rat[14] and to be incorporated into the nucleic acid of tumors[15] suggested the desirability of preparing a number of 9-aryl-2,6-diaminopurines which as nucleoside models might exhibit anti-tumor activity. For preparation of the desired 2,6-diaminopurines (XIII), 6-arylamino-2,4,5-triaminopyrimidine (XII) was needed as the requisite intermediate. Attempts to follow the general route employed in the synthesis of the 9-arylguanines (IV) was unsuccessful due to the difficulties encountered in the attempted preparation of 6-chloro-2,4-diamino-5-phenylazopyrimidine by coupling 6-chloro-2,4-diaminopyrimidine (IX) and phenyldiazonium chloride. The alternative procedure of preparing the required 6-arylamino-2,4-diaminopyrimidine (X) from 6-chloro-2,4-diaminopyrimidine (IX) proceeded smoothly in an aqueous alcoholic solu-



REACTION SCHEME II

tion in the presence of a catalytic amount of hydrochloric acid. The aminolysis of a 4-chloropyrimidine with an aromatic amine has previously been shown to proceed readily in the presence of acid.[16] The 6-arylamino-2,4-diaminopyrimidines (X) were readily nitrosated to give the corresponding 5-nitroso derivative XI which in turn was reduced with sodium hydrosulfite to yield the 5-aminopyrimidine (XII). Purification of XII was not attempted, but the tetraaminopyrimidine was treated directly with formamide to give the desired 2,6-diamino-9-phenylpurine. The 9-aryl-2,6-diaminopurines thus prepared are listed in Table IV. It is interesting to note that the cyclization of XII with formamide gave the 2,6-diamino-9-phenyl-

(12) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **77**, 1676 (1955).

(13) J. H. Burchenal, A. Bendich, G. B. Brown, G. B. Elion, G. H. Hitchings, C. P. Rhoads and C. C. Stock, *Cancer*, **2**, 119 (1949).

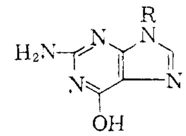
(14) A. Bendich, S. S. Furst and G. B. Brown, *J. Biol. Chem.*, **185**, 423 (1950).

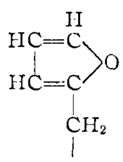
(15) L. L. Bennett, H. F. Skipper, H. W Toolan and C. P. Rhoads, *Cancer Research*, **16**, 262 (1956).

(16) (a) C. K. Banks, THIS JOURNAL, **66**, 1127 (1944); (b) A. Maggiolo and A. P. Phillips, *J. Org. Chem.*, **16**, 376 (1951).

TABLE II

9-ALKYL(ARYL)-2-AMINO-6-HYDROXYPURINES



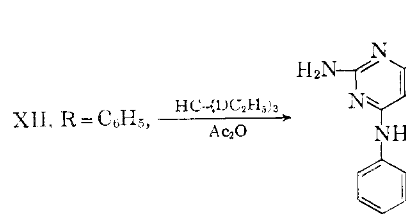
R[a]	Over-all yield from 1, %	M.p.,[b] °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	λmax, mμ pH 1	ε	λmax, mμ pH 11	ε
$C_6H_5CH_2$	29	300–302	59.8	59.6	4.8	4.6	29.0	28.9	255 280	13,100 8,500	270	11,900
3,4-$Cl_2C_6H_3CH_2$	26	342–343	46.2	45.7	3.3	3.7	22.4	22.5	255 280	13,100 9,400	270	12,500
o-$ClC_6H_4CH_2$	31	335–336	52.2	52.2	3.6	3.5	25.4	25.5	255 280	14,900 10,000	270	12,700
n-$CH_3(CH_2)_9$	41	233–234	61.8	61.9	8.6	8.9	24.0	24.0	255 280	16,300 11,100	270	11,600
n-$CH_3(CH_2)_{10}$	52	234–236	63.0	63.1	8.8	9.1	23.0	23.1	255 280	10,400 6,700	270	10,100
p-$ClC_6H_4CH_2$	25	343–344 d.	52.2	52.5	3.6	3.9	25.4	25.5	255 280	14,600 9,700	270	13,300
Cyclo-C_6H_{11}	34	>360	56.7	56.4	6.4	6.3	30.0	29.9	255 280	10,700 7,500	270	12,100
	34[c]	306–307 d.	52.0	51.8	3.9	3.9	30.4	30.2	255 280	12,700 8,300	270	10,400
i-C_5H_{11}	32	352 d.	54.4	54.4	6.4	6.6	31.6	31.5	255 280	12,200 8,200	270	12,400
n-C_8H_{17}	41	282–283	59.3	59.3	8.0	8.1	26.6	26.8	255 280	12,200 7,800	270	11,200
n-C_6H_{13}	43	283–284	56.2	56.1	7.2	7.1	29.8	29.6	255 280	13,400 8,900	270	11,800
C_6H_5	43	>360	58.2	58.4	3.9	4.0	30.8	31.0	262	12,000	268	12,500
p-BrC_6H_4	36	>360	43.2	43.5	2.8	2.5	22.8	22.8	229 262	19,600 10,700	244 266	15,300 12,800
p-ClC_6H_4	38	>360	50.5	50.5	3.4	3.2	26.8	26.8	263	12,500	268 234	14,600 20,700
i-C_4H_9[10]	34	>360	52.5	52.2	6.3	6.7	33.8	33.8	255 280	12,400 8,100	270	10,800
$CH_2CH_2C_6H_5$	34	323–324	60.7	60.5	5.1	5.2	27.0	26.6	255 280	12,200 8,000	270	11,200
$C_{10}H_7$	35	>360	66.1	66.0	4.0	4.2	25.7	25.9	255 280	9,000 6,500	270	8,100

[a] Compounds recrystallized from N,N-dimethylformamide. [b] Melting points >300° determined on copper block and are uncorrected. [c] Recrystallized from dilute acetic acid.

purine (XIII) exclusively. This is not unexpected since a similar cyclization of 6-anilino-4,5-diaminopyrimidine with formamide has previously been reported[17] to give 9-phenyladenine. The cyclization of 6-anilino-2,4,5-triaminopyrimidine with triethyl orthoformate and acetic anhydride proceeded to give the isomeric 2-amino-6-anilinopurine (XV) exclusively. The structures, XIII, R = C_6H_5, and XV, were differentiated on the basis of the fact that the latter compound was soluble in sodium hydroxide due to the presence of the imidazole hydrogen and gave an insoluble silver salt with silver nitrate in dilute sulfuric acid.

(17) J. W. Daly and B. E. Christensen, *J. Org. Chem.*, **21**, 177 (1956).

Treatment of 6-anilino-2,4,5-triaminopyrimidine (XII, R = C_6H_5) with sodium nitrite in acetic acid gave 5,7-diamino-3-phenyl-v-triazolo(d)pyrimidine

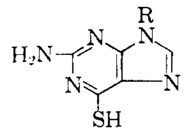


XV

(XIV, R = C_6H_5). The structure XIV was assigned since the product was insoluble in strong

TABLE III

9-ALKYL(ARYL)-2-AMINO-6-PURINETHIOLS

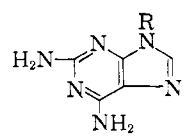


R[a]	Over-all yield from IX, %	M.p.,[b] °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	λmax, mμ pH 1	ε	λmax, mμ pH 11	ε
C_6H_5	52	304–305	54.4	54.1	3.8	3.6	28.9	29.1	343	27,600	320	24,800
p-ClC_6H_4	31	308–310	47.8	47.7	2.9	2.9	25.2	24.9	343	16,800	320	14,500
p-BrC_6H_4	34	310–312	41.8	41.6	2.5	2.7	21.7	21.7	233	20,200	237	22,200
									342	15,500	318	13,500
n-$CH_3(CH_2)_3$	30	316–318	58.6	58.4	8.1	8.0	22.8	22.7	262	6,100	250	11,700
									343	14,700	270	6,700
											320	17,200
$C_6H_5CH_2$	35	303–304	56.0	55.8	4.7	4.5			262	9,100	275	13,500
									343	22,100	320	19,500

[a] Compounds recrystallized from N,N-dimethylformamide. [b] Melting points determined on copper block and are uncorrected.

TABLE IV

9-ARYL-2,6-DIAMINOPURINES



R	Over-all yield from IX, %	M.p.,[a] °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	λmax, mμ pH 1	ε	λmax, mμ pH 11	ε
C_6H_5	35	283–285	58.4	58.7	4.4	4.1	37.1	37.4	230	26,000	280	12,400
									291	10,800		
p-ClC_6H_4	31	304–305	50.7	50.9	3.4	3.6	32.3	32.8	240	20,400	279	14,200
									293	9,900		
3,4-$Cl_2C_6H_3$	26	304–305	44.8	44.9	2.7	2.7	28.5	28.6	237	28,200	280	14,700
									290	12,300		
p-BrC_6H_4	39.6	315–317	43.2	43.3	2.9	2.9	27.5	27.7	240	28,600	278	15,200
									292	12,800		
p-$CH_3C_6H_4$	42	292–293	60.0	60.0	5.0	4.8	35.0	35.5	230	28,800	280	13,400
									290	12,000		

[a] Melting points >300° determined on copper block and are uncorrected.

potassium hydroxide solution. Hull[18] has recently reported that similar treatment of 4,5-diamino-6-furfurylaminopyrimidine and nitrous acid gives 7-amino-3-furfuryl-v-triazolo(d)pyrimidine. The ultraviolet absorption spectra of the purines and v-triazolo(d)pyrimidines have been recorded at pH 1 and 11.

Experimental[18a]

Preparation of 4-Alkyl(aryl)amino-2-amino-6-hydroxy-5-phenylazopyrimidines (II).—To 250 ml. of absolute alcohol, containing 0.1 mole of the appropriate amine, was added 25 g. (0.1 mole) of 2-amino-4-chloro-6-hydroxy-5-phenylazopyrimidine (I).[5] This solution was refluxed for 5 hr. A complete solution occurred after just a few minutes of reflux, and then the desired product began to crystallize from the reaction mixture. The solution was cooled and filtered, and the product was washed with alcohol and then with ether. Several attempts to recrystallize these 5-phenylazopyrimidines were unsuccessful.

2-Amino-4-(p-chloroanilino)-6-hydroxy-5-phenylazopyrimidine (II, R = p-ClC_6H_4) was prepared in 96% yield. At pH 1 it exhibited λ_{max} 257 mμ, ϵ 17,400, and λ_{max} 425 mμ, ϵ 22,200; at pH 11, λ_{max} 281 mμ, ϵ 14,600, and λ_{max} 392 mμ, ϵ 15,300.

Anal. Calcd. for $C_{16}H_{13}N_6OCl \cdot H_2O$: N, 23.4. Found: N, 23.6.

2-Amino-4-(p-bromoanilino)-6-hydroxy-5-phenylazopyrimidine (II, R = p-BrC_6H_4) was obtained in 90% yield. It did not melt below 300° and exhibited at pH 1, λ_{max} 257 mμ, ϵ 16,900, and λ_{max} 425 mμ, ϵ 21,000; at pH 11, λ_{max} 283 mμ, ϵ 11,600, and λ_{max} 393 mμ, ϵ 11,600.

Anal. Calcd. for $C_{16}H_{13}N_6OBr \cdot H_2O$: N, 20.8. Found: N, 21.2.

2-Amino-4-anilino-6-hydroxy-5-phenylazopyrimidine (II, R = C_6H_5).—When the 4-alkyl(aryl)amino-2-amino-6-hydroxy-5-phenylazopyrimidines (II) did not crystallize from the alcoholic solution upon cooling, water was added to the reaction mixture to precipitate the product. By this procedure 2-amino-4-anilino-6-hydroxy-5-phenylazopyrimidine (II, R = C_6H_5) was prepared in 78% yield. A small amount of crude product was reprecipitated from hot, dilute sodium hydroxide with acetic acid for analysis. It did not melt below 300° and exhibited at pH 1, λ_{max} 256 mμ, ϵ 16,200, and λ_{max} 425 mμ, ϵ 21,800; at pH 11, λ_{max} 277 mμ, ϵ 15,600, and λ_{max} 393 mμ, ϵ 16,800.

Anal. Calcd. for $C_{16}H_{14}N_6O \cdot H_2O$: N, 25.9. Found: N, 25.6.

4-Alkyl(aryl)amino-2,5-diamino-6-hydroxypyrimidine (III).—To approximately 25 g. of the crude 4-alkyl(aryl)-

(18) R. Hull, *J. Chem. Soc.*, 2746 (1958).

(18a) Melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected, unless otherwise indicated.

amino-2-amino-6-hydroxy-5-phenylazopyrimidine (II), dissolved in 600 ml. of 2.5 N sodium hydroxide, was added 75 g. of sodium hydrosulfite. The solution was gently boiled 10 min. during which time it changed from dark red to light yellow. The solution was finally treated simultaneously with Norite and Filter-cel to absorb the oily aniline. The clear filtrate was then neutralized with acetic acid and cooled. The white solid was filtered and washed with a small amount of water and used in the next step. Attempts to purify this material further resulted in discoloration and decomposition of the product.

9-Alkyl(aryl)-2-amino-6-hydroxypurines (IV).—The crude 4-alkyl(aryl)amino-2,5-diamino-6-hydroxypyrimidine (III), obtained from 25 g. of II, was added to 100 ml. of C.P. formamide. The solution was boiled gently for 30 min. and then diluted with 500 ml. of water and cooled to yield the crude 2-amino-6-hydroxy-9-substituted purine. Purification was effected by dissolving the crude material in dilute hydrochloric acid followed by precipitation from the hot solution with ammonium hydroxide. Final recrystallization was accomplished from N,N-dimethylformamide or an aqueous N,N-dimethylformamide mixture. The over-all yields of the 2-amino-6-hydroxy-9-substituted purines thus obtained are recorded in Table II.

9-Alkyl(aryl)-2-amino-6-purinethiols (VIII).—Ten grams of 9-alkyl(aryl)-2-amino-6-hydroxypurine (VII) and 30 g. of phosphorus pentasulfide was ground together in a mortar and then transferred to a flask containing 500 ml. of A.C.S. grade pyridine. The mixture was refluxed from 4 to 24 hr. depending upon the solubility of the starting material in the pyridine. At the end of this time the excess pyridine was distilled under reduced pressure, and 400 ml. of water was added to the residue. This mixture was placed on the steam-bath for 2 hr. to ensure decomposition of excess phosphorus pentasulfide. At the end of this time the product was filtered and washed with an abundance of water and then alcohol. For purification the compound was reprecipitated from hot, dilute potassium hydroxide with acetic acid and finally recrystallized from an N,N-dimethylformamide–water solution to give light-yellow crystals.

5-Amino-7-hydroxy-3-substituted-v-triazolo(d)pyrimidines (V).—The crude 4-alkyl(aryl)amino-2,5-diamino-6-hydroxypyrimidine (III), obtained from 25 g. of II, was added to 250 ml. of water. Just enough sodium hydroxide was added to effect solution. A small amount of insoluble material (probably sulfur) was filtered off, and 10 g. of sodium nitrite was added to the solution. After acidification with glacial acetic acid the solution was heated on the steam-bath for 2 hr. then cooled and filtered to yield the desired product. For purification the crude compound was reprecipitated from dilute base with acetic acid and finally recrystallized from N,N-dimethylformamide.

2,6-Dihydroxy-9-phenylpurine (VI, R = C_6H_5).—Three grams of 2-amino-6-hydroxy-9-phenylpurine (IV, R = C_6H_5) was added to 150 ml. of boiling water. To this mixture was added enough concentrated hydrochloric acid to dissolve the purine and 10 ml. in excess. The hot solution was mechanically stirred and maintained at 90° while a solution of 3 g. of sodium nitrite in 20 ml. of water was slowly added dropwise. Stirring was continued for 15 min. after addition. The solution was chilled, and the product was filtered and recrystallized from dilute acetic acid to yield 1.5 g. of 2,6-dihydroxy-9-phenylpurine. The ultraviolet spectra of the compound was identical to that of a sample of the compound synthesized earlier by a different method.[10]

2,6-Dihydroxy-9-*n*-undecylpurine (VI, R = n-$C_{11}H_{25}$).—Five grams of 2-amino-6-hydroxy-9-n-undecylpurine (IV, R = $C_{11}H_{25}$) was treated with nitrous acid as for the preparation of 2,6-dihydroxy-9-phenylpurine (VI, R = C_6H_5) to yield 3.5 g. of 2,6-dihydroxy-9-n-undecylpurine (VI, R = n-$C_{11}H_{25}$). The product was recrystallized from dilute acetic acid to yield shiny, colorless crystals, m.p. > 300°.

Anal. Calcd. for $C_{16}H_{26}N_4O_2$: C, 62.7; H, 8.5; N, 18.3. Found: C, 62.9; H, 8.6; N, 18.5.

3-Benzyl-5,7-dihydroxy-v-triazolo(d)pyrimidine (VII, R = $CH_2C_6H_5$).—One gram of 5-amino-3-benzyl-7-hydroxy-v-triazolo(d)pyrimidine (VI, R = $CH_2C_6H_5$) was added to 200 ml. of boiling water. Ten ml. of concentrated hydrochloric acid was added, and the solution was heated to boiling. To the stirred solution was added dropwise a solution of 1 g. of sodium nitrite in 10 ml. of water. Stirring and heating were continued for 30 min. The solution was then treated with Norite and filtered. The cooled filtrate yielded 0.8 g. of 3-benzyl-5,7-dihydroxy-v-triazolo(d)pyrimidine (VII, R = $CH_2C_6H_5$) as light-yellow, shiny plates, m.p. > 300°.

Anal. Calcd. for $C_{11}H_9N_5O_2$: C, 54.4; H, 3.7; N, 28.8. Found: C, 54.0; H, 3.7; N, 28.7.

2,6-Diamino-4-substituted-aminopyrimidines (X).—To a solution of 75 ml. of water and 50 ml. of alcohol was added 1.5 ml. of concentrated hydrochloric acid, 0.1 mole of the appropriate aniline and 14.4 (0.1 mole) of 4-chloro-2,6-diaminopyrimidine (IX).[19] The solution was then refluxed for 4 hr. and poured into 400 ml. of boiling water. The resulting solution was treated with Norite, made slightly alkaline with ammonium hydroxide and cooled to yield the desired product.

4-Anilino-2,6-diaminopyrimidine (X, R = C_6H_5).—Aniline (9.3 g.) allowed to react with IX in the above manner yielded 14 g. of 4-anilino-2,6-diaminopyrimidine (X, R = C_6H_5). Recrystallization from water yielded colorless plates, m.p. 182–184°.

Anal. Calcd. for $C_{10}H_{11}N_5$: N, 34.8. Found: N, 35.1.

4-*p*-Chloroanilino-2,6-diaminopyrimidine (X, R = p-Cl C_6H_4).—p-Chloroaniline (12.7 g.) treated with IX in the above manner yielded 18 g. of 4-p-chloroanilino-2,6-diaminopyrimidine (X, R = p-C_6H_4). Recrystallization from dilute acetic acid yielded light-yellow crystals, m.p. 168–170°.

Anal. Calcd. for $C_{10}H_{10}N_5Cl$: N, 29.7. Found: N, 29.8.

2,6-Diamino-4-*p*-toluidinopyrimidine (X, R = p-CH_3-C_6H_4).—p-Toluidine (10.7 g.) reacted similarly yielding 24 g. of 2,6-diamino-4-p-toluidinopyrimidine (X, R = p-CH_3-C_6H_4). Recrystallization from benzene yielded plates, m.p. 170–172°.

Anal. Calcd. for $C_{11}H_{14}N_5$: N, 32.5. Found: N, 32.6.

4-Anilino-2,6-diamino-5-nitrosopyrimidine (XI).—The 4-anilino-2,6-diaminopyrimidine (X) (0.1 mole) was dissolved in 250 ml. of 10% acetic acid. The solution was cooled to 10° and stirred mechanically while 0.1 mole of sodium nitrite in 75 ml. of water was added dropwise. Stirring was continued for 1 hr., then the crude, highly-colored nitroso-derivative was filtered, washed with ice-water and reduced directly to the corresponding 4-anilino-2,5,6-triaminopyrimidine (XII).

4-Anilino-2,5,6-triaminopyrimidine Bisulfite (XII).—The crude 4-anilino-2,6-diamino-5-nitrosopyrimidine (XI) was suspended in 500 ml. of boiling water. To the suspension was added 75 g. of sodium hydrosulfite, and the resulting solution was allowed to boil 10 min., then chilled. The crude bisulfite salt was filtered, washed with a little ice-water, sucked dry and immediately carried on to the next step.

2,6-Diamino-9-substituted Purines (XIII).—The crude 2,5,6-triamino-4-substituted-aminopyrimidine (XII), prepared previously, was suspended in 100 ml. of C.P. formamide and boiled gently for 30 min. At the end of this time the solution was diluted with 300 ml. of water and then cooled. The crude product was filtered, washed with water, dissolved in 250 ml. of dilute hydrochloric acid and boiled with Norite. The cooled filtrate deposited the hydrochloride salt of the appropriate 2,6-diaminopurine. For analysis a small amount of the hydrochloride was changed to the free base with ammonium hydroxide.

2-Amino-6-anilinopurine (XV).—Ten grams of 4-anilino-2,5,6-triaminopyrimidine bisulfite (XII, R = C_6H_5) was converted to the free pyrimidine with dilute ammonium hydroxide. The pyrimidine was washed with acetone and dried in a vacuum desiccator. This process was accompanied by a substantial amount of discoloration. The pyrimidine was then added to 250 ml. of a 1:1 mixture of ethyl orthoformate and acetic anhydride, and the solution was refluxed for 8 hr. The reaction mixture was cooled and filtered, and the product was washed with water. The crude product was then treated with 250 ml. of boiling 2 N sodium hydroxide. Neutralization of the solution with acetic acid yielded 3.5 g. of long, white needles upon cooling. Recrystallization from N,N-dimethylformamide–water yielded needles, m.p. 283–285°. This compound exhibited at pH 1 λ_{max} 300 mμ, ϵ 21,900; at pH 11, λ_{max} 304 mμ, ϵ 21,400, and λ_{max} 237 mμ, ϵ 20,700.

Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 58.4; H, 4.4; N, 37.1. Found: C, 58.7; H, 4.6; N, 37.3.

(19) Purchased from Francis Earle Laboratories, Peekskill, N. Y.

5,7-Diamino-3-phenyl-v-triazolo(d)pyrimidine (XIV, R = C_6H_5).—Fifteen grams of the 4-anilino-2,5,6-triaminopyrimidine bisulfite (XII) was dissolved in 300 ml. of boiling water. To this solution was added 50 ml. of glacial acetic acid, and the solution was stirred while 10 g. of sodium nitrite in 100 ml. of water was slowly added. The solution was then heated on the steam-bath for 1 hr. and cooled. The resulting precipitate was filtered and washed with water. For analysis the compound was recrystallized from N,N-dimethylformamide to yield 4.5 g. of 5,7-diamino-3-phenyl-v-triazolo(d)pyrimidine (XIV, R = C_6H_5), m.p. >300°. This product was insoluble in aqueous potassium hydroxide solution.

Anal. Calcd. for $C_{10}H_9N_7$: C, 52.5; H, 3.6; N, 43.1. Found: C, 52.8; H, 3.9; N, 43.5.

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Synthesis of Peptides Related to Gramicidin S. III.[1] The Decapeptide Containing L-Lysine Residues in Place of L-Ornithine[2]

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The synthesis of a decapeptide analog of gramicidin S is described. It differs from the latter in being acyclic and containing two L-lysine residues instead of two L-ornithines. It was obtained in crystalline form as were fourteen of the fifteen polypeptide intermediates, adsorption chromatography being utilized for the purification of the higher intermediates.

This paper reports further progress in the synthesis of decapeptide analogs of gramicidin S. The polypeptide described in this paper is the decapeptide H·Val-Lys-Leu-Phe-Pro-Val-Lys-Leu-Phe-Pro·OH·3HCl(L-L-L-D-L)$_2$[3](I).

The structure of gramicidin S and the decapeptides synthesized to date are shown in Fig. 1; gramicidin S is a cyclic decapeptide containing a repeated sequence of five amino acid residues. Two of the component amino acid residues, L-ornithine and D-phenylalanine, are not frequently encountered in naturally occurring polypeptides and appear rarely, if at all, in proteins. It is the purpose of this program to ascertain which parts of the chemical structure of gramicidin S are responsible for its antibiotic activity. This objective is being pursued by means of the synthesis and antibacterial assay of various decapeptide analogs.

It was reported earlier[4] that decapeptide (II)[1] possessed antibacterial activity, although it was less potent than gramicidin S. It was proposed that its lower activity might be the result of its greater susceptibility to bacterial hydrolytic enzymes because of its acyclic structure. The suggestion was made that the cyclic structure of gramicidin S, though not necessary for its antimicrobial activity, prevents destruction of the peptide by the microörganism. Antibacterial studies of decapeptides I, II and III and several to be prepared will test this hypothesis and perhaps establish the chemical structure responsible for the bactericidal properties of gramicidin S.

It should be noted here that Schwyzer and Sieber[5] have recently synthesized gramicidin S, utilizing a pentapeptide intermediate described in paper I[1] of this series.

The synthetic methods used to prepare the decapeptide I are described in Fig. 2. As emphasized in previous papers, choice of synthetic techniques was governed by the necessity of preventing diastereoisomer formation. For this reason, the azide route was employed in all cases where acylated peptides served as intermediates. Fourteen of the fifteen compounds were obtained in crystalline form, a positive demonstration of the efficacy of the azide method for the preparation of complex polypeptides.

Val-Orn-Leu-Phe-Pro-Val-Orn-Leu-Phe-Pro
(L-L-L-D-L)$_2$ Gramicidin S

H·Val-Lys-Leu-Phe-Pro-Val-Lys-Leu-Phe-Pro·OH
(L-L-L-D-L)$_2$ (I)

H·Val-Orn-Leu-Phe-Pro-Val-Orn-Leu-Phe-Pro·OH
(L-L-L-D-L) (II)

H·Val-Orn-Leu-Tyr-Pro-Val-Orn-Leu-Tyr-Pro·OH
(L-L-L-D-L) (III)

Fig. 1.—Synthetic peptides.

As in the preparation of decapeptide III (ref. 1, paper II), it was necessary to perform the synthesis by the reaction of a tetrapeptide, Z·Val-*p*-Tos·Lys-Leu-Phe·NH·NH$_2$(L-L-L-D) with a hexapeptide, H·Pro-Val-*p*-Tos·Lys-Leu-Phe-Pro·OMe(L-L-L-L-D-L). Decapeptide II (ref. 1, paper I) was synthesized by the reaction of two pentapeptide derivatives, but this scheme was not feasible here because Z·Val-*p*-Tos·Lys-Leu-Phe-Pro·NH·NH$_2$(L-L-L-D-L) could not be obtained in pure crystalline form.

The pentapeptide, H·Val-*p*-Tos·Lys-Leu-Phe-Pro·OMe·HCl (L-L-L-D-L)(compd. 11) was found to crystallize in two forms, as needles and as rhombohedra, depending upon the quantity of methanol in the recrystallizing solvent.

(1) Paper I: B. F. Erlanger, H. Sachs and E. Brand, THIS JOURNAL, **76**, 1806 (1954); paper II: B. F. Erlanger, W. V. Curran and N. Kokowsky, *ibid.*, **80**, 1128 (1958).

(2) This research is supported by the Office of Naval Research under contract N-onr-266(44). A preliminary account appears in the Abstracts of the 133rd American Chemical Society meeting, San Francisco, Calif., April, 1958, p. 27-C.

(3) For an explanation of the abbreviations, see papers I and II (ref. 1). Briefly: Z, carbobenzyloxy, $C_6H_5CH_2OCO$; *p*-Tos, *p*-toluenesulfonyl, $C_7H_7SO_2$; Leu, leucyl, $NH(CHC_4H_9)CO$; Val, valyl, $NH(CHC_3H_7)CO$; etc. The configurations of the amino acid residues appear in parentheses after the name of the compound.

(4) B. F. Erlanger and L. Goode, *Nature*, **174**, 840 (1954).

(5) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 624 (1957).