Purine Sulfonamides

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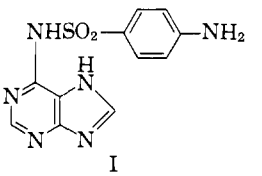
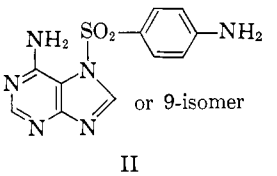
A number of purine sulfonamides have been synthesized mostly by the reaction of a chloropurine with sodium sulfanilamide. The compounds exhibit little or no chemotherapeutic activity.

Despite the widespread use of several pyrimidine sulfonamide drugs, information on analogous compounds carrying a purine nucleus is scanty and contradictory. Since both purines and pyrimidines occur in living cells, it appeared logical to attach a purine moiety to a sulfa drug in the hope of obtaining sulfonamides with desirable properties. The synthesis of these compounds is the subject of the present communication.

A few sulfonamide derivatives of the methylated xanthines have been described[1] and the rather ambiguous "sulfanilyl nucleic acid" has been claimed in a patent.[2] Of more interest is N^1-6-purinylsulfanilamide (I). Several workers[3] have reported the synthesis of I from adenine and *p*-acetylaminobenzenesulfonyl chloride or *p*-nitrobenzenesulfonyl chloride followed by hydrolysis or reduction, respectively. The compound obtained from reduction of the nitro derivative is described by Berlin and Sjögren[3a] as melting at 258–259° dec and as being difficulty soluble in dilute sodium hydroxide. Lack of solubility in dilute base, however, would not be expected for a substance possessing structure I. It is interesting to note that in a patent[3b] Sjögren claims that the product obtained from adenine and *p*-acetylaminobenzenesulfonyl chloride, upon heating with 10% NaOH, gave "sulfanilyladenine" which gave a correct analysis for sulfur, while in an article[3a] Berlin and Sjögren state that upon hydrolysis of 6-acetylsulfanilamidopurine with 10% sodium hydroxide, "a product was obtained which did not contain sulfur but which had the same melting point as purine." (Could purine be a misprint for 6-aminopurine or adenine?) Jensen and Falkenberg[4] note that the product from adenine and *p*-acetylaminobenzenesulfonyl chloride is split with hot NaOH to adenine and acetylsulfanilic acid and suggest that the initial product is 6-amino-7- (or 9-) acetylsulfanilylpurine, while the compound with mp 258–259° dec obtained by reduction of the corresponding nitro compound,[3a] is 6-amino-7- (or 9-) sulfanilylpurine (II).



I



II

We have shed a new light on the problem by the unambiguous synthesis of I from 6-chloropurine and sodium sulfanilamide. The N^1-6-purinylsulfanilamide so obtained melts at 363–364° dec and is readily soluble in dilute sodium hydroxide. It is therefore not identical with the product of mp 258–259°.

The presence of methoxy groups and halogen in some of the active pyrimidine sulfonamides[5] suggested their incorporation into the purine analogs. It was also felt that introduction of methyl groups in the 7 or 9 position and avoidance of free hydroxy groups or combinations of hydroxy and amino groups, which tend to decrease solubility[6] of the purines, would be beneficial.

From 2,6-dichloro-9-methylpurine[7] (III), N^1-(2-chloro-9-methyl-6-purinyl)sulfanilamide (IV) was readily obtained by heating with sodium sulfanilamide in dimethylformamide. The structure of IV was proven by hydrogenation to N^1-(9-methyl-6-purinyl)-sulfanilamide (V) prepared independently from 6-chloro-9-methylpurine[8] (VI) and sodium sulfanilamide (Scheme I). Replacement of the more reactive 6-chlorine atom in 2,6-dichloro-9-methylpurine was to be expected from the work of Fischer.[9] Similarly 2,6-dichloro-7-methylpurine[10] (VII) gave N^1-(2-chloro-7-methyl-6-purinyl)sulfanilamide (VIII) which was hydrogenated to N^1-(7-methyl-6-purinyl)sulfanilamide (IX) and also converted to N^1-(2-methoxy-7-methyl-6-

(1) (a) I. Satoda, T. Fukui, Y. Matsuo, and H. Okumura, *Yakugaku Kenkyu*, **28**, 621 (1956); *Chem. Abstr.*, **51**, 16494*g* (1957); (b) H. Morishita, S. Nakano, I. Satoda, N. Yoshida, T. Fukui, Y. Matsuo, and H. Okumura, Japanese Patent 535 (1959); *Chem. Abstr.*, **54**, 6767*b* (1960); (c) P. Pon, Belgian Patent 451,086 (1943); *Chem. Abstr.*, **42**, 215*c* (1948).

(2) S. L. Ruskin, U. S. Patent 2,407,686 (1946); *Chem. Abstr.*, **41**, 1393*b* (1947).

(3) (a) E. Berlin and B. Sjögren, *Svensk. Kem. Tidskr.*, **53**, 457 (1941); *Chem. Abstr.*, **37**, 3744[9] (1943); (b) B. Sjögren, Swedish Patent 106,964 (1943); *Chem. Zentr.*, II, 2253 (1943); (c) A. R. Frisk, *Acta Med. Scand. Suppl.*, **142**, 1 (1943); *Chem. Abstr.*, **38**, 4692[8] (1944); (d) R. Plasseraud, French Patent 872,799 (1942); *Chem. Zentr.*, II, 1319 (1943); (e) K. Ganapathi, *Current Sci.* (India), **9**, 457 (1940); *Brit. Chem. Physiol. Abstr.*, *A2*, 109 (1941).

(4) K. A. Jensen and P. Falkenberg, *Dansk Tidsskr. Farm.*, **16**, 141 (1942); *Chem. Zentr.*, I, 626 (1943).

(5) For example: N^1-(2,4-dimethoxy-6-pyrimidinyl)sulfanilamide, Madribon®, N^1-(2-methyl-4-methoxy-6-pyrimidinyl)sulfanilamide, N^1-(4-methoxy-6-pyrimidinyl)sulfanilamide, and N^1-(5-methoxy-2-pyrimidinyl)sulfanilamide (see L. Neipp in "Experimental Chemotherapy," Vol. II, R. J. Schnitzer and F. Hawking, Ed., Academic Press Inc., New York, N. Y., 1964, p 179) and N^1-(5-bromo-4,6-dimethyl-2-pyrimidinyl)sulfanilamide (see the "Merck Index," 7th ed, Merck and Co., Inc., Rahway, N. J., 1960, p 992).

(6) The solubilities of many purines are given by A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

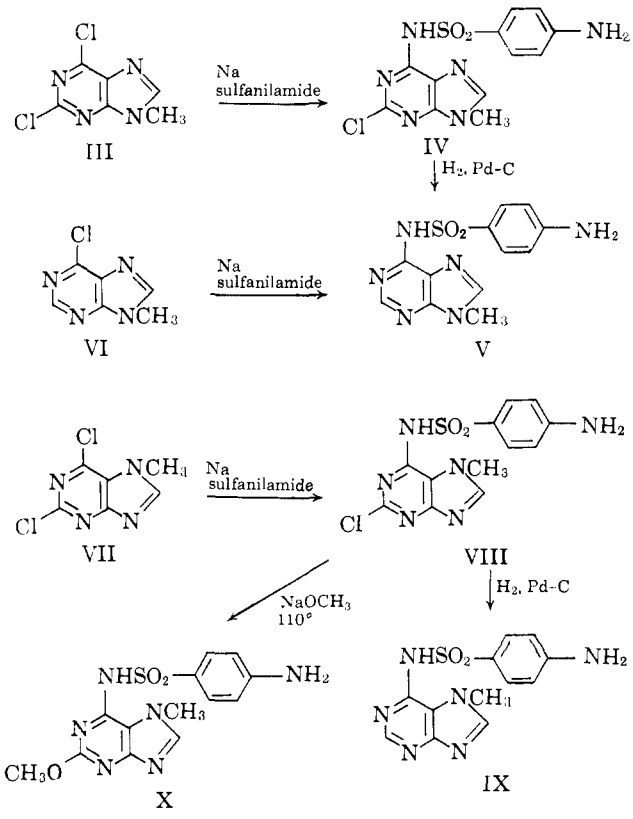
(7) A. G. Beaman and R. K. Robins, *J. Org. Chem.*, **28**, 2310 (1963).

(8) R. K. Robins and H. H. Lin, *J. Am. Chem. Soc.*, **79**, 490 (1957).

(9) E. Fischer, *Ber.*, **30**, 2227 (1897).

(10) G. Y. Uretskaya, E. I. Rybkina, and G. P. Menshikov, *Zh. Obshch. Khim.*, **30**, 327 (1960).

Scheme I



purinyl)sulfanilamide (X) by treatment with methanolic $NaOCH_3$ at 110°.

Treatment of 9-methyl-2,6,8-trichloropurine[11] (XI) with sodium sulfanilamide gave N^1-(2,6-dichloro-9-methyl-8-purinyl)sulfanilamide (XII) the structure of which was proven by hydrogenation to N^1-(9-methyl-8-purinyl)sulfanilamide (XIII) which was synthesized independently from 8-chloro-9-methylpurine[7] (XIV) and sodium sulfanilamide. Nucleophilic substitution of 7- or 9-methyl-2,6,8-trichloropurine usually occurs first at position 8. However, some of the 6 isomer is generally formed as well. This was first observed by Fischer[12] and recently confirmed by Sutcliffe and Robins.[11] The formation of isomers may explain the low yield of pure XII. Upon treatment with methanolic sodium methoxide at 110° XII gave N^1-(2,6-dimethoxy-9-methyl-8-purinyl)sulfanilamide (XV), whereas at room temperature N^1-(2-chloro-6-methoxy-9-methyl-8-purinyl)sulfanilamide (XVI) was formed (Scheme II). The structure of XVI is assumed on the basis of the greater activity of a chlorine atom at position 6 as compared to position 2 in purines.[9] Similarly N^1-[2-chloro-6-(β-methoxyethoxy)-9-methyl-8-purinyl]-sulfanilamide (XX) was formed from XII and a solution of sodium in β-methoxyethanol at room temperature. From 2-chloro-9-methylpurine[7] (XVII) and sodium sulfanilamide only a very small yield of N^1-(9-methyl-2-purinyl)sulfanilamide (XVIII) was obtained. Thus a better route to XVIII was sought. Partial hydrogenation of nonalkylated polychloropurines has been described.[13] We have applied this method to 2,6-dichloro-9-methylpurine[7] (III) which gave 2-chloro-9-methylpurine (XVII), identical with the compound previously obtained by methylation of 2-chloropurine.[7] This constitutes a more convenient route to XVII. Although several attempts to convert 2-chloropurines to 2-aminopurines even under drastic conditions have failed,[14] treatment of XVII with methanolic ammonia containing NH_4Cl[15] at 125° gave 2-amino-9-methylpurine (XIX). Treatment of XIX with *p*-acetylaminobenzenesulfonyl chloride followed by hydrolysis gave N^1-(9-methyl-2-purinyl)sulfanilamide (XVIII). The ultraviolet absorption spectra and pK_a values of several purine sulfonamides are given in Table I.

Chemotherapy. Materials and Methods.—The bacteriostatic effect *in vitro* was determined by the conventional technique of the serial-dilution test in the semisynthetic medium of Adams and Roe.[16] The inoculum consisted of 0.05 ml of a 10^{-3} diluted overnight broth culture except in the case of streptococci and pneumococci where the same volume of a 10^{-1} diluted culture was employed. The drugs were dissolved or suspended in water. The results were read after 24 hr. incubated, and verified by subculture on appropriate solid media if necessary.

For determination of acute toxicity mice weighing 18–20 g received a single dose of the purine sulfonamide under test. After a 72-hr observation period the LD_{50} values were calculated by the method of Reed and Muench.[17]

All experiments to determine antibacterial activity *in vivo* were carried out in white mice of 18–20 g. The animals were infected intraabdominally with 100–1000 minimal lethal doses of the bacterial strain to be tested and treated either subcutaneously or orally according to procedures previously described.[18,19] The 50% end point (CD_{50}) based on survivors and/or negative cultures was calculated according to the method of Reed and Muench.

Results.—The data in Table II indicate that all purine sulfonamide derivatives tested were well tolerated by the subcutaneous, oral and/or intraperitoneal routes. The data on the bacteriostatic activity *in vitro*, which are given in Table III showed that little or no effect was exhibited by the purine sulfonamides tested against *Streptococcus hemolyticus*, *Staphylococcus aureus*, and *Diplococcus pneumoniae*. Against *Escherichia coli* and *Salmonella typhi* marked activity was noted with V. Moderate activity was noted when IV was tested against *E. coli*. The remaining substances showed only slight or no effect.

Four of the purine sulfonamides were studied for their capacity of reversal by *p*-aminobenzoic acid

(11) E. V. Sutcliffe and R. K. Robins, *J. Org. Chem.*, **28**, 1662 (1963).

(12) (a) E. Fischer, *Ber.*, **28**, 2490 (1895); (b) *ibid.*, **30**, 1846 (1897); (c) *ibid.*, **31**, 104 (1898); (d) *ibid.*, **32**, 267 (1899).

(13) (a) S. R. Bresbeers, S. S. Wang, S. G. Beebtolt, and P. E. Christenson, *J. Am. Chem. Soc.*, **81**, 3789 (1959); (b) H. Ballweg, *Ann.*, **649**, 114 (1961); (c) H. Bredereck, H. Herlinger, and I. Graudums, *Chem. Ber.*, **95**, 54 (1962).

(14) (a) R. R. Adams and F. C. Whitmore, *J. Am. Chem. Soc.*, **67**, 1271 (1945); (b) J. A. Montgomery and L. B. Holum, *ibid.*, **79**, 2185 (1957); (c) *ibid.*, **80**, 404 (1958).

(15) The role of ammonium chloride in catalyzing the replacement of halogen by ammonia is discussed by G. Spielberger in Houben-Weyl, "Methoden der Organischen Chemie," Vol. 11/1, Eugen Müller, Ed., 4th ed, Georg Thieme Verlag, Stuttgart, 1957, pp 30–31.

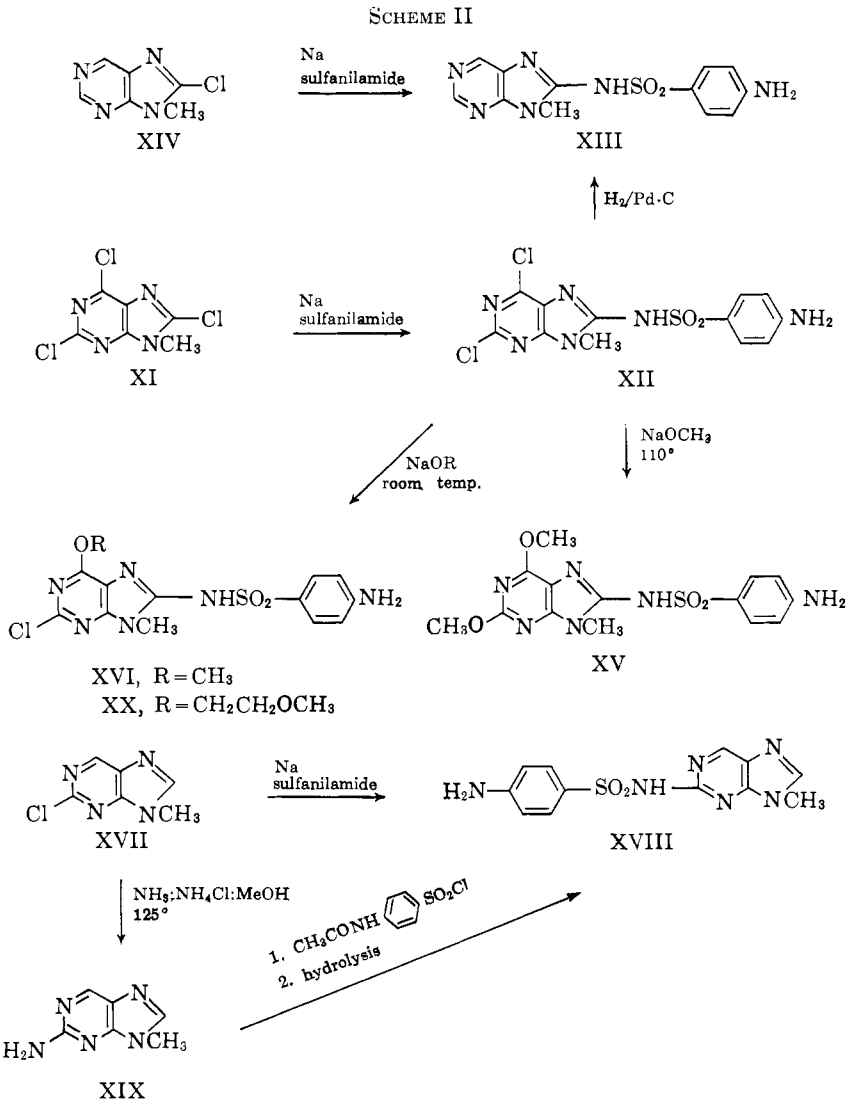
(16) M. H. Adams and A. S. Roe, *J. Bacteriol.*, **49**, 401 (1945).

(17) L. J. Reed and J. Muench, *Am. J. Hyg.*, **27**, 493 (1938).

(18) E. Grunberg, L. O. Randall, and R. J. Schnitzer, *J. Pharmacol. Exptl. Therap.*, **95**, 336 (1949).

(19) W. F. DeLorenzo and E. Grunberg in "Antimicrobial Agents and Chemotherapy—1963," J. C. Sylvester, Ed., Braun-Brumfield, Inc., Ann Arbor, Mich., 1964, pp 550–553.

SCHEME II



(PABA). From Table IV it may be noted that a significant reversal of activity by PABA was seen with V, the only substance which showed marked activity *in vitro*. The remaining three substances tested for this effect were antagonized to a lesser extent.

None of the substances, except XVIII, showed any effect against the *S. hemolyticus* No. 4, *S. aureus* Smith, *E. coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *S. typhi* infections of mice. This compound showed slight activity against *S. typhi*, the CD_{50} being 250 mg/kg *po*.

Pharmacology.—Compounds IV, V, VIII, IX, and XX were tested as blood sugar lowering agents. Blood sugar determinations were made on blood samples taken at 2 and 4 hr from the tail and analyzed by the Autotechnicon method. They were inactive in modifying the blood sugar of rats when administered orally in doses of 100–200 mg/kg to groups of five fasted rats.

Experimental Section[20]

N^1-6-Purinylsulfanilamide (I).—To a stirred suspension of 15.5 g (0.08 mole) of sodium sulfanilamide in 75 ml of dimethylformamide (DMF) which was heated to 95° there was added 10 g (0.065 mole) of 6-chloropurine.[21] The solids dissolved. The mixture was stirred at 93–102° for 2 hr, cooled to 9°, diluted with 150 ml of cold water and acidified to pH 3.8[22] by addition of 60 ml of 1 *N* HCl. After refrigeration overnight a brown gelatinous solid (2.2 g) was removed by filtration. The filtrate was evaporated, and the solid residue was slurried with 150 ml of acetone, filtered, and washed with two 25-ml portions of acetone. The product (16.8 g) was powdered finely, slurried with 1.2 l. of water and dissolved by addition of 95 ml of 1 *N* NaOH. After treatment with 3.5 g of Norit A the solution was acidified to pH 3.5 with 93 ml of 1 *N* HCl. The precipitated solid was dried and leached with 100 ml of boiling ethanol to give 9.0 g (48%) of I, mp 363–364° dec.

Anal. Calcd for $C_{11}H_{10}N_6O_2S$: C, 45.50; H, 3.47; N, 28.95; S, 11.04. Found: C, 45.61; H, 3.60; N, 28.91; S, 10.72.

N^1-(2-Chloro-9-methyl-6-purinyl)sulfanilamide (IV).—To a stirred suspension of 76 g (0.392 mole) of sodium sulfanilamide in 428 ml of DMF which was heated to 75° there was added 61.1 g (0.301 mole) of 2,6-dichloro-9-methylpurine.[7] The resulting solution was stirred at 83–87° for 1.5 hr, cooled to 8°, diluted with 850 g of a water–ice mixture, and acidified to pH 2.7 with 215 ml of 1 *N* HCl. The product which precipitated was dissolved in 800 ml of acetone. After removal of a little insoluble material by filtration, the solution was evaporated to 50 ml to give 46.8 g of crystals (washed with a little cold acetone). Upon addition of 2.5 l. of water to the original reaction mixture an additional 14.6 g of product was obtained. The combined crude

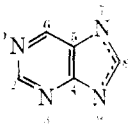
(20) The purity of the compounds was checked by thin layer chromatography with silica gel and mixtures of chloroform or benzene and methanol or acetone. All melting points were taken on a Thomas–Hoover melting point apparatus and are uncorrected.

(21) (a) A. Bendich, P. Russell, Jr., and J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954); (b) A. G. Beaman and R. K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).

(22) The acidification should be done without delay, or the product cannot be isolated.

TABLE I

ULTRAVIOLET ABSORPTION AND pK_a VALUES OF SOME PURINE SULFONAMIDES



	Position					$\lambda_{max}^{pH\ 1-2}$,		$\lambda_{max}^{pH\ 12}$,		
No.	2	6	8	7	9	mμ	ε	mμ	ε	pK_a
I	H	Sul[a]	H	H	...	311	24,700	306	32,300	9.4[b]
VIII	Cl	Sul	H	CH_3	...	290	19,900	256	15,200	Approx 4[c]
								292	22,000	
IX	H	Sul	H	CH_3	...	293	22,800	256	17,800	5.9[b]
								287	24,400	
X	OCH_3	Sul	H	CH_3	...	247	7,100	252	17,000	5.55[b]
						292	23,300	295	22,900	5.4[c]
IV	Cl	Sul	H	...	CH_3	281	15,700	258	16,000	5.3[d]
								289	25,800	4.3[c]
V	H	Sul	H	...	CH_3	287	24,000	256–260[e]	18,700	5.95[b]
								281	28,500	6.7[d]
XII	Cl	Cl	Sul	...	CH_3	302	28,800	258	19,300	4.2[c]
								312	21,300	
XIII	H	H	Sul	...	CH_3	294	22,500	258	19,300	5.95[b]
								297	19,500	
XVI	Cl	OCH_3	Sul	...	CH_3	286	28,000	260	20,600	5.5[b]
								291	24,500	5.5[c]
XV	OCH_3	OCH_3	Sul	...	CH_3	236–241[e]	9,500	254	22,900	6.7[b]
						287	23,000	288	20,000	6.7[c]
XVIII	Sul	H	H	...	CH_3	228–235[e]	18,500	227	27,800	6.7[c]
						256–265[e]	9,800	255	19,800	7.9[d]
								291–300[e]	7,600	
XX	Cl	$OCH_2CH_2OCH_3$	Sul	...	CH_3	287	28,300	261	20,700	
								292	24,000	

[a] Sul = p-$H_2NC_6H_4SO_2NH$. [b] Determined by trituration in water. [c] Determined spectrophotometrically. [d] Determined by titration in 48% DMSO in water. [e] Shoulder.

TABLE II

ACUTE TOXICITY OF PURINE SULFONAMIDES IN MICE

	LD_{50}, mg/kg		
Compd	Sc	Po	Ip
IX	>500	>1000	>1000
X	>500	>500	>500
IV	>500	>2000	>2000
V	>500	>2000	>2000
XII	...	1682	1320
XIII	...	>500	>500

TABLE III

In Vitro ANTIBACTERIAL ACTIVITY OF PURINE SULFONAMIDES

	Min inhib concn (μg/ml) in Adams and Roe broth against				
Compd	*S. hemolyticus*	*D. pneumoniae*	*S. aureus*	*E. coli*	*S. typhi*
PABA	>1000	>1000	>1000	>1000	>1000
IX	>5000	>5000	1250	156	625
X	2500	2500	625	312.5	>5000
IV	2500	2500	1250	39.0	312.5
V	>5000	>5000	625	9.75	9.75
XII	>5000	2500	1250	>5000	>5000
XIII	>5000	>5000	625	625	>5000

material was recrystallized from 9 l. of boiling ethanol to give 51.0 g (50%) of product (needles), mp 254.5–255° dec.

Anal. Calcd for $C_{12}H_{11}ClN_6O_2S$: C, 42.54; H, 3.23; N, 24.81. Found: C, 43.13; H, 3.68; N, 24.50.

N^1-(9-Methyl-6-purinyl)sulfanilamide (V). A.—A solution of 12.0 g (35.4 mmoles) of N^1-(2-chloro-9-methyl-6-purinyl)sulfanilamide (IV) in 165 ml of distilled water and 72 ml of 1 N NaOH was hydrogenated in a Parr apparatus under 4.22 kg/cm² pressure in the presence of 3.0 g of 10% Pd–C catalyst. Hydrogen uptake ceased after 15–20 min. After removal of the catalyst the solution was acidified to pH 3 with 1 N HCl (about 40 ml) and refrigerated to yield 8.60 g (80%) of crystals, mp 254–255°. A sample was recrystallized rapidly from 100 parts of water.

Anal. Calcd for $C_{12}H_{12}N_6O_2S$: C, 47.36; H, 3.97; N, 27.62. Found: C, 47.75; H, 4.33; N, 27.28.

B.—A slurry of 0.64 g (3.3 mmoles) of sodium sulfanilamide in 15 ml of DMF was stirred at 95° with 0.50 g (3.0 mmoles) of 6-chloro-9-methylpurine[8] for 2 hr. The solid obtained by evaporation of the reaction mixture was triturated with about 4 ml of water. A solid, mp 159–162°, was removed by filtration. Gradual addition of 20 ml of acetone to the filtrate gave crystals which were filtered and dissolved in a small volume of water. Acidification to pH 6 with HCl gave material: mp 247–248°; $\lambda_{max}^{pH\ 1}$ 287 mμ; ratio of optical densities, 287 mμ/240 mμ = 4.95; $\lambda_{max}^{pH\ 12}$ 256–260 mμ (sh), 281 mμ; ratio of optical densities 281 mμ/260 mμ = 1.44, in agreement with the spectrum of V (Table I) prepared by A.

N^1-(2-Chloro-7-methyl-6-purinyl)sulfanilamide (VIII).—2,6-Dichloro-7-methylpurine[10] (86 g, 0.424 mole) was treated with sodium sulfanilamide (123 g, 0.634 mole) in DMF (430 ml) at 90–95° (2.75 hr). After cooling a solid (no ultraviolet absorption) was removed and the filtrate was evaporated *in vacuo* to

TABLE IV

COMPARISON OF THE *in Vitro* ANTIBACTERIAL ACTIVITY OF PURINE SULFONAMIDES IN ADAMS AND ROE BROTH WITH AND WITHOUT PABA

	Min inhib concn (μg/ml) in Adams and Roe broth					
	——Without PABA——			Containing 1000 μg/ml of PABA		
Compd	*S. aureus*	*E. coli*	*S. typhi*	*S. aureus*	*E. coli*	*S. typhi*
IX		625	1250		2500	2500
X	625	312.5		5000	2500	
IV		78	312.5		625	1250
V		3.9	15.6		>500	>500

a paste. This was stirred with 1 l. of acetone for 1 hr and filtered. The acetone-insoluble solid was stirred with 2.5 l. of water for 1 hr and filtered (solid discarded), and the filtrate was acidified to pH 2.5 with 1 *N* HCl to give 60 g (42%) of crude product. This was dissolved in 2.5 l. of water containing 165 ml of 1 *N* NaOH and reprecipitated by slow acidification (pH 2.4) with 1 *N* HCl to give 51 g of purified material. A sample was recrystallized from 300 parts of water (charcoal).

Anal. Calcd for $C_{12}H_{11}ClN_6O_2S$: C, 42.54; H, 3.23; N, 24.81. Found: C, 42.22; H, 3.38; N, 24.79.

N¹-(7-Methyl-6-purinyl)sulfanilamide (IX).—N¹-(2-Chloro-7-methyl-6-purinyl)sulfanilamide (VIII$_1$, 12 g, 35.4 mmoles) was hydrogenated as described for IV for 3 hr, and the product was isolated as previously described to give 8.4 g (78%) of nearly white solid. Recrystallization from 8.5 parts of water gave an 81% recovery of crystals, mp 232–233.5°.

Anal. Calcd for $C_{12}H_{12}N_6O_2S$: C, 47.36; H, 3.97; N, 27.62. Found: C, 47.79; H, 4.26; N, 27.63.

N¹-(2-Methoxy-7-methyl-6-purinyl)sulfanilamide (X).—A mixture of 4.00 g (11.8 mmoles) of N¹-(2-chloro-7-methyl-6-purinyl)sulfanilamide (VIII), 100 ml of absolute methanol, and 20 ml of a 25% solution of $NaOCH_3$ in methanol was heated at 110° in a rocking autoclave for 6 hr. After cooling, a small amount of insoluble material was removed by filtration, the filtrate was evaporated to dryness, and the residue was dissolved in 175 ml of water. The pH of the filtrate was adjusted to 3.8 with HCl and the gummy solid which precipitated was recrystallized three times from methanol to give 1.42 g (36%) of crystals, mp 205–206°.

Anal. Calcd for $C_{13}H_{14}N_6O_3S$: C, 46.70; H, 4.22; N, 25.14. Found: C, 46.68; H, 4.40; N, 25.21.

N¹-(2,6-Dichloro-9-methyl-8-purinyl)sulfanilamide (XII).—To a stirred suspension of 75 g (0.386 mole) of sodium sulfanilamide in 750 ml of DMF which was heated to 55°, there was added 75 g (0.316 mole) of 9-methyl-2,6,8-trichloropurine.[11] The mixture was stirred at 62–65° for 1.5 hr, cooled, and filtered. To the stirred filtrate was added a mixture of 800 ml of water and ice and 137 ml of 1 *N* HCl. Stirring was continued for 30 min, and the solid which formed was collected. It was washed with water, slurried with 500 ml of acetone, filtered, and washed with acetone. It was dissolved in 5.5 l. of water containing 150 ml of 1 *N* NaOH and treated with 15 g of Norit A. To the filtrate was added 1 *N* HCl (35–40 ml) until a permanent haze formed. The hazy solid was filtered and the stirred filtrate was acidified (pH 3.5) by the slow addition of about 110 ml of 1 *N* HCl to give 42.4 g of crude product. This was recrystallized twice from 200 parts of toluene (Norit A) to give colorless needles, mp 239.5–240° dec, which were dried at 100° (0.3 mm) to remove toluene of crystallization; yield, 20.2 g (17%).

Anal. Calcd for $C_{12}H_{10}Cl_2N_6O_2S$: C, 38.62; H, 2.70; Cl, 19.00. Found: C, 38.89; H, 2.92; Cl, 18.92.

N¹-(9-Methyl-8-purinyl)sulfanilamide (XIII). A.—A solution of 6.0 g (16 mmoles) of thoroughly purified N¹-(2,6-dichloro-9-methyl-8-purinyl)sulfanilamide (XII) in 152 ml of water and 48 ml of 1 *N* NaOH was hydrogenated in a Parr apparatus (3.52 kg/cm² of hydrogen, 2.3 g of 10% Pd–C). The reaction was complete after 13 min. The catalyst was filtered, and the filtrate was acidified with 1 *N* HCl to pH 3.5 and cooled. The colorless crystalline solid was collected, washed with water, and dried; mp 250–251°, yield 4.12 g (85%) of analytically pure product.

Anal. Calcd for $C_{12}H_{12}N_6O_2S$: C, 47.37; H, 3.98; N, 27.62; O, 10.52; S, 10.54. Found: C, 47.43; H, 4.13; N, 27.68; O, 10.89; S, 10.56.

B.—A mixture of 25 ml of DMF, 0.69 g (3.6 mmoles) of sodium sulfanilamide, and 0.50 g (3.0 mmoles) of 8-chloro-9-methylpurine[7] was stirred at 86–93° for 2 hr. The reaction mixture was cooled to 5°, and a mixture of 50 ml of water and ice, plus 2 ml of 1 *N* HCl was added (pH 4). Evaporation of the solution gave a sticky solid which was leached with 40 ml of acetone. The filtrate was evaporated to 5 ml to give a product still contaminated with some sulfanilamide. Recrystallization from 25 ml of ethanol gave nearly colorless needles, mp 247–248°, showing no depression when mixed with material prepared by A. Samples prepared by A and B also gave identical ultraviolet and infrared spectra and R_f values on tlc developed with $CHCl_3$–methanol (80:20).

N¹-(2,6-Dimethoxy-9-methyl-8-purinyl)sulfanilamide (XV).—A solution of 1.00 g (2.68 mmoles) of analytically pure N¹-(2,6-dichloro-9-methyl-8-purinyl)sulfanilamide (XII) and 1.43 g of $NaOCH_3$ in 100 ml of absolute methanol was autoclaved for 6 hr at 110° under nitrogen. The residue obtained upon evaporation of the solvent was dissolved in 50 ml of water, and the solution was neutralized to pH 7.0 with dilute HCl to give 0.49 g (50% crude yield) of tan solid. This was recrystallized twice from methanol to give colorless crystals, mp 281° dec.

Anal. Calcd for $C_{14}H_{16}N_6O_4S$: C, 46.55; H, 4.93; OCH_3, 17.03. Found: C, 45.93; H, 4.83; OCH_3, 16.89.

N¹-(2-Chloro-6-methoxy-9-methyl-8-purinyl)sulfanilamide (XVI).—One gram (2.68 mmoles) of thoroughly purified N¹-(2,6-dichloro-9-methyl-8-purinyl)sulfanilamide (XII) was dissolved in 55 ml of absolute methanol containing 8.05 mmoles of $NaOCH_3$. The solution, after standing at room temperature for 24 hr, was acidified to pH 3.2 by gradual addition of 65 ml of 0.1 *N* HCl. The resulting crystalline solid was collected, washed with water, and dried to yield 0.75 g of product. Recrystallization from 75 ml of ethanol gave (in two crops) 0.47 g (48%) of material, mp 270° dec.

Anal. Calcd for $C_{13}H_{13}ClN_6O_3S$: C, 42.34; H, 3.55; Cl, 9.61. Found: C, 42.54; H, 3.93; Cl, 9.73.

2-Chloro-9-methylpurine (XVII).—A solution of 2,6-dichloro-9-methylpurine[7] (18.4 g, 90.7 mmoles) in 1.26 l. of ethanol was mixed with a solution of 27.5 g of sodium acetate trihydrate in 450 ml of water. This was hydrogenated at atmospheric pressure in the presence of 3.3 g of 10% Pd–C catalyst. In 10 min 2.32 l. STP (1 mole + 10%) of hydrogen was absorbed. The filtrate from the catalyst was evaporated *in vacuo*. The resulting solid was slurried with 50 ml of distilled water at room temperature, filtered, and washed with 10 ml of water to give 13.3 g (87%) of crude XVII, mp 120–130°. (The crude material which was difficult to purify completely could be aminated directly to the much more readily purified 2-amino-9-methylpurine.) A portion was recrystallized from benzene until the melting point was raised to 132°. It was identical with an authentic sample[7] of 2-chloro-9-methylpurine as judged by melting point, mixture melting point, and ultraviolet and infrared spectra.

2-Amino-9-methylpurine (XIX).—Crude 2-chloro-9-methylpurine (13.3 g, 79 mmoles) was dissolved in 1.2 l. of 4 *N* NH_3 in methanol and 30.2 g of NH_4Cl was added. The mixture was heated at 125–130° in a rocking autoclave for 20 hr under nitrogen (28.12 kg/cm²). The filtrate was evaporated, and the resulting yellow solid was crystallized from 140 ml of water to give 4.79 g (41%) of needles, mp 242–243°. For the analysis a portion was sublimed (0.2 mm, 140–150°) to give a product: mp 242–243°; $\lambda_{max}^{pH\ 1}$ 248, 314 mμ (ε 3600, 4000); λ_{max}^{pH12} 242, 302 mμ (ε 5500, 8000).

Anal. Calcd for $C_6H_7N_5$: C, 48.31; H, 4.73; N, 46.96. Found: C, 48.48; H, 4.56; N, 47.22.

N¹-(9-Methyl-2-purinyl)sulfanilamide (XVIII). A.—To a solution of 2-amino-9-methylpurine (XIX, 4.79 g, 32.1 mmoles) in 250 ml of pyridine at 75° was added 15.1 g (64.6 mmoles) of freshly recrystallized *p*-acetylaminobenzenesulfonyl chloride. After heating at 70–72° for 1 hr, the pyridine was removed *in vacuo* to give a syrup which was slurried three times with water, and the water was removed *in vacuo* each time. The resulting yellow solid was triturated with 100 ml of water to give 7.41 g of the acetylated XVIII. It was hydrolyzed by heating at 100° with 34 ml of 2 *N* HCl for 30 min. The filtered solution was adjusted to pH 5 by addition of aqueous NH_3 and cooled to give 5.45 g of crude XVIII. Two crystallizations from 1:1 ethanol–water (charcoal) gave 2.64 g (27%) of nearly colorless crystals, mp 270–271°.

Anal. Calcd for $C_{12}H_{12}N_6O_2S$: C, 47.37; H, 3.98; N, 27.62; S, 10.54. Found: C, 47.73; H, 4.14; N, 27.31; S, 10.52.

B.—A stirred mixture of 20 ml of DMF, 2.23 g (12 mmoles) of sodium sulfanilamide, and 1.04 g (6 mmoles) of pure 2-chloro-9-methylpurine was refluxed for 3 hr, cooled to 10°, and acidified to pH 5 by addition of a mixture of 40 ml of water and 8 ml of 1 *N* HCl. The solution was evaporated at room temperature to a gum which was triturated with 10 ml of acetone and filtered. The filtrate was diluted with 30 ml of ethanol. After removal of tarry material by filtration, the solution was evaporated to 4 or 5 ml to give about 0.2 g of yellow solid. Recrystallization from ethanol gave an off-white solid, mp 270–271°, which had the same infrared and ultraviolet spectra as the material prepared by A.

N¹-[2-Chloro-6-(β-methoxyethoxy)-9-methyl-8-purinyl]sulfanilamide (XX).—Throughly purified N¹-(2,6-dichloro-9-methyl-8-purinyl)sulfanilamide (XII, 1.50 g, 4.02 mmoles) was added to a cooled solution of 0.28 g (12.06 mg-atoms) of Na in 50 ml of

2-methoxyethanol. After 19 hr at room temperature, the solution was diluted to about 300 ml with water and acidified to pH 2.9 by gradual addition of 1 *N* HCl. After cooling, the solid was collected and washed with water, to yield 1.26 g (76%) of XX, mp 232–236° dec. A sample was recrystallized from ethanol to give pale yellow flakelets, mp 228–229° dec.

Anal. Calcd for $C_{15}H_{17}ClN_6O_3S$: C, 43.64; H, 4.15; Cl, 8.59; N, 20.36. Found: C, 43.57; H, 4.44; Cl, 8.66; N, 20.47.

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Synthetic Schistosomicides. VIII. N-Mono- and N,N-Dialkyl-N′-(4-arylazo-1-naphthyl)alkylenediamines and Related Compounds[1]

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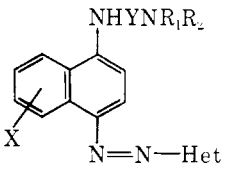
Several hundred N-mono- and N,N-dialkyl-N′-(4-arylazo-1-naphthyl)alkylenediamines (III) were synthesized by (1) coupling a diazotized arylamine with the appropriate 1-(aminoalkyl)naphthylamine, (2) amination of a N-(ω-haloalkyl)-4-(arylazo)-1-naphthylamine, and (3) hydrolysis of N-(aminoalkyl)-N-[4-(arylazo)-1-naphthyl]-2,2,2-trifluoroacetamides or formamides. Schistosomicidal activity among the N,N-dialkyl-N′-(4-arylazo-1-naphthyl)alkylenediamines is widespread, and twenty-nine compounds cured *Schistosoma mansoni* infections in mice at doses ranging from 78 to 734 mg/kg per day for 14 days. Six compounds were evaluated against *S. mansoni* infections in rhesus monkeys and each showed significant antischistosomal activity in this host. Structure–activity relationships are discussed.

During the course of continuing efforts in these laboratories to develop novel schistosomicidal agents, it was discovered that various [4-(dialkylaminoalkylamino)-1-naphthylazo]heterocyclic compounds (I) possess strong therapeutic activity against *Schistosoma*

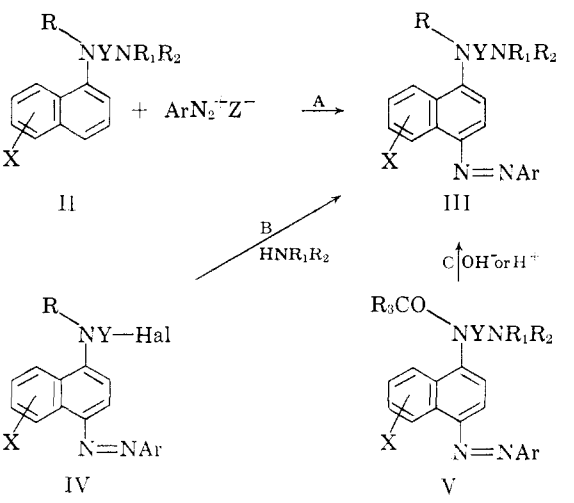


I

mansoni in experimental animals.[2,3] We have been actively engaged in extending this work to other series[1,4–8] and now wish to report the synthesis of a group of N-mono- and N,N-dialkyl-N′-(4-arylazo-1-naphthyl)alkylenediamines (III, where R, R_1, and R_2 represent a hydrogen atom or an alkyl group, Y an alkylene radical, X a hydrogen or halogen atom or a hydroxy or alkoxy group, and Ar a phenyl or naphthyl radical), many of which exhibit remarkable schistosomicidal activity in mice. After the completion of this work, the synthesis of several azo derivatives of 1-diethylamino-3-(1-naphthylamino)-2-propanol was reported.[9]

Three major routes (Chart I) were utilized in the preparation of the N-mono- and N,N-dialkyl-N′-(4-arylazo-1-naphthyl)alkylenediamines (III) (Tables

CHART I



I–V): (1) coupling a diazotized arylamine with the appropriate 1-(aminoalkyl)naphthylamine (II)[10] (route A) (procedures I–IV); (2) amination of a N-(ω-haloalkyl)-4-(arylazo)-1-naphthylamine (IV) with the appropriate amine (route B) (procedure VI); and (3) hydrolysis of an N-(aminoalkyl)-N-[4-(arylazo)-1-naph-

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