250 ml. of saturated ethanolic ammonia was heated in an autoclave at 135° for 12 hr. The resulting solution was evaporated to dryness and the residue recrystallized from butanol. A final recrystallization from aqueous ethanol afforded an analytically pure product.

The structural assignment (see Discussion part) in this group of compounds was further substantiated by paper chromatographic measurements (one spot in each case). R_f values for 2,4-diamino-6-(*p*-bromoanilino)-5-pyrimidinecarbonitrile: 0.71 (isopropyl alcohol–water, 7:3), and 0.80 (butanol saturated with acetic acid); for 2,4-diamino-6-(*p*-iodoanilino)-5-pyrimidinecarbonitrile: 0.73 and 0.81, respectively.

2,4-Diamino-6-(substituted-anilino)-5-pyrimidinecarboxamide (Table I). General Procedure.—With good stirring, 2,4-diamino-6-(substituted-anilino)-5-pyrimidinecarbonitrile (0.05 mole) was added slowly to 50 ml. of concentrated sulfuric acid at 30–35°. The solution was stirred at this temperature for 12 hr. and then added, with stirring, to 500 g. of flaked ice. The precipitated product was separated by filtration and washed well with ice–water. The crude product was dissolved in hot, dilute sulfuric acid, treated with charcoal, and filtered. The filtrate was adjusted to pH 8 with aqueous ammonia and the product filtered while hot, washed with water, and dried at 100°. Repeated reprecipitation afforded product of analytical purity.

4,6-Dichloro-2-methylaminopyrimidine.—In a flask equipped with two modified Friedrichs condensers designed to retain low boiling liquids was added 23 g. (0.23 mole) of triethylamine and 7 g. (0.23 mole) of methylamine diluted with 200 ml. of ethyl acetate. To this solution was added 30 g. (0.154 mole) of 4,6-dichloro-2-(methylsulfonyl)pyrimidine[15] dissolved in 200 ml. of ethyl acetate. The temperature was kept below 35° during the entire reaction. After 2 hr. of stirring the reaction mixture was evaporated and the pale yellow residue recrystallized from ethanol to give 13 g. (77%) of white crystals, m.p. 162–163°. The product was found to be identical with that reported by Winkelmann[14a] and Boon.[14b]

4-Amino-6-chloro-2-methylaminopyrimidine.—A mixture of 4,6-dichloro-2-methylaminopyrimidine (10.8 g.) and ethanolic ammonia was heated at 80° for 8 hr. in a sealed vessel. Evaporation of the reaction mixture and purification of the product from methanol afforded 7 g. (65%) of white crystals, m.p. 193–194°.

Anal. Calcd. for $C_5H_7ClN_4 \cdot H_2O$: N, 31.8. Found: N, 31.9.

General Preparations of 4-(Substituted-anilino)-5-nitrosopyrimidines (V). A.—A mixture of the appropriate 4-chloropyrimidine, an equivalent amount (plus a 10% excess) of the substituted aniline, and several milliliters of concentrated hydrochloric acid was heated in an oil bath. A clear, dark colored melt was observed around 150° followed by an exothermic reaction that caused the temperature to rise to *ca.* 200°. The reaction mixture was held at this temperature for several minutes. It was then cooled, dissolved in boiling ethanol, treated with charcoal, and filtered. The filtrate was made basic with dilute aqueous ammonia and cooled. The precipitated 4-(substituted-anilino)pyrimidine was filtered and washed with water. Nitrosation of the intermediate to the desired product was carried out by the procedure of O'Brien, *et al.*[2]

B.—A solution of 0.10 mole of the appropriate 4-chloropyrimidine, 0.11 mole of the substituted aniline, and 2 ml. of concentrated hydrochloric acid in 200 ml. of 50% ethanol was refluxed for 4 hr. The reaction mixture was poured into 800 ml. of boiling water. The resulting solution was treated with charcoal, filtered, and the filtrate adjusted to pH 8 with aqueous ammonia. On cooling it deposited the intermediate 4-(substituted-anilino)-pyrimidine, which was then isolated and nitrosated as described in Method A.

Acknowledgment.—The authors wish to express their appreciation to Mrs. Phyllis G. Lewis, Mrs. Margaret L. Rounds, and Mr. Hal P. Van Fossen for their valuable assistance in performing analytical and instrumental measurements.

Synthesis and Antitumor Activity of 9-(Tetrahydro-2-furyl)purine Analogs of Biologically Important Deoxynucleosides[1]

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Received March 11, 1963

The syntheses of the 9-(tetrahydro-2-furyl) derivatives of hypoxanthine, guanine, and 2-amino-6-purinethiol (6-thioguanine) have been accomplished. The reaction of 2,3-dihydro-2-methylfuran with 6-chloropurine has been studied. Several of the 9-(tetrahydro-2-furyl)purines exhibit significant antitumor activity against a variety of experimental mouse tumors. The significance of these results is discussed in terms of therapeutic index, transport, and structural relationship to various purine-2′-deoxynucleosides and other biologically active purine derivatives.

The synthesis of certain 6-substituted-9-(tetrahydro-2-pyranyl)purines and 6-substituted-9-(tetrahydro-2-furyl)purines has recently been reported.[2,3] The preliminary antitumor activity of several of these derivatives has prompted a detailed study of this group of compounds. Since the 9-(tetrahydro-2-furyl)purines appeared to be superior in the early stages of animal testing, attention was directed to these derivatives. Because the 9-(tetrahydro-2-furyl)purines can be envisaged as analogs of the important naturally occurring purine deoxynucleosides, the first major goal of this work was to synthesize the 9-(tetrahydro-2-furyl) analog of 2′-deoxyguanosine and 2′-deoxyinosine. 6-Amino-9-(tetrahydro-2-furyl)purine, the analog of 2′-deoxyadenosine, had previously been prepared[3] and exhibited definite antitumor activity. An attempt to prepare 6-hydroxy-9-(tetrahydro-2-furyl)purine (IV) directly from 6-chloro-9-(tetrahydro-2-furyl)purine with aqueous sodium hydroxide resulted in degradation of the purine ring to give 6-chloro-4,5-diaminopyrimidine[4] as the only isolatable product. In order to obtain IV, 6-benzyloxypurine (I) was prepared from 6-chloropurine by the general method of Huber.[5] Treatment of I with 2,3-dihydrofuran in the presence of acid gave

(1) This investigation was supported by Research Grants CY-4008(C3) and CY-4008(C4) from the National Cancer Institute of the National Institutes of Health, Public Health Service, Bethesda 14, Md.

(2) R. K. Robins, E. F. Godefroi, E. C. Taylor, L. R. Lewis, and A. Jackson, *J. Am. Chem. Soc.*, **83**, 2574 (1961).

(3) L. R. Lewis, F. H. Schneider, and R. K. Robins, *J. Org. Chem.*, **26**, 3837 (1961).

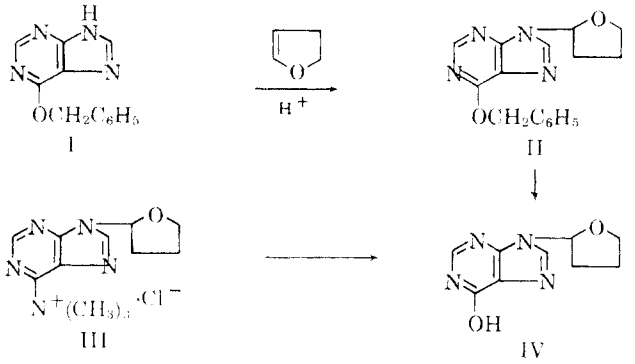
(4) R. K. Robins, K. J. Dille, C. H. Willits, and B. E. Christensen, *J. Am. Chem. Soc.*, **75**, 263 (1953).

(5) G. Huber, *Chem. Ber.*, **90**, 698 (1957).

6-benzyloxy-9-(tetrahydro-2-furyl)purine (II), which was debenzylated catalytically with palladium-on-carbon to give the desired compound, 6-hydroxy-9-(tetrahydro-2-furyl)purine (IV), the analog of 2′-deoxyinosine. It was later discovered that trimethyl-[9-(tetrahydro-2-furyl)-6-purinyl]ammonium chloride (III),[3] in the presence of very dilute base, gave IV directly in good yield. Since the structure of III had been previously established as 9-substituted, it follows that IV prepared from II is indeed 6-hydroxy-9-(tetrahydro-2-furyl)purine because an identical product was obtained by both methods. Further proof of structure of IV was obtained from ultraviolet absorption studies. 9-Methylhypoxanthine[6] in ethanol shows λ_{max} at 249 mμ. 7-Methylhypoxanthine[7] in ethanol exhibits λ_{max} at 257 mμ. The 9-(tetrahydro-2-furyl) derivative (IV) exhibits a λ_{max} of 248 mμ in ethanol which is indicative of substitution at position 9 instead of 7.

The synthesis of 9-(tetrahydro-2-furyl)purines possessing a substituent other than hydrogen at position 2 has not been reported previously. Thus, the reaction of 2,6-dichloropurine[8] and 2,3-dihydrofuran was investigated. A good yield of 2,6-dichloro-9-(tetrahydro-2-furyl)purine (V) was readily obtained. The structure of V was certain since the ultraviolet absorption spectrum of 2,6-dichloro-7-methylpurine[9,10] in ethanol is λ_{max} 282 mμ; 2,6-dichloro-9-methylpurine[11,12] exhibits λ_{max} of 274 mμ in ethanol. 2,6-Dichloro-9-(tetrahydro-2-furyl)purine (V) in ethanol showed λ_{max} 274 mμ.

Reaction Scheme I

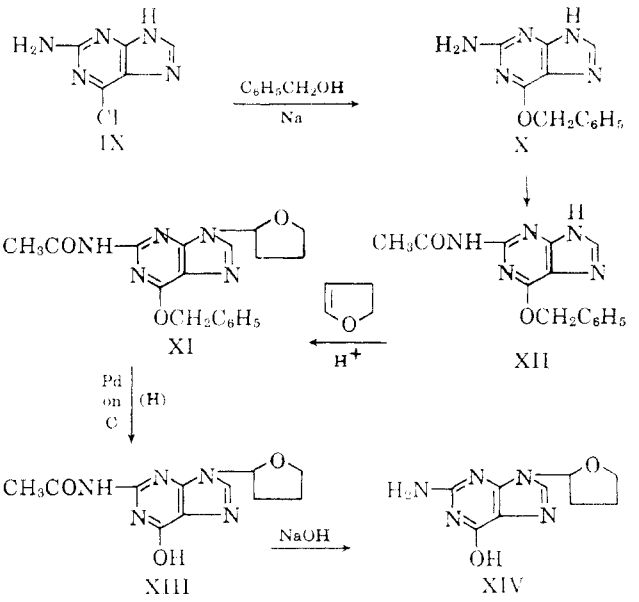


Treatment of V with aqueous methylamine gave 2-chloro-6-methylamino-9-(tetrahydro-2-furyl)purine (VII). Methanolic ammonia at room temperature and V led to 6-amino-2-chloro-9-(tetrahydro-2-furyl)purine (VI). Treatment of VI with sodium methoxide in methanol furnished 6-amino-2-methoxy-9-(tetrahydro-2-furyl)purine (VIII). Compound VIII is of interest because of its structural relationship to the nucleoside, spongosine,[13] (6-amino-2-methoxy-9-β-D-ribofuranosylpurine) isolated from a Caribbean sponge.

The attachment of the tetrahydrofuran ring to the purine nucleus *via* 2,3-dihydrofuran has been shown[3] to proceed best when electron-withdrawing substituents are in the purine ring. A mechanism which is in accord with this experimental observation has been proposed.[3] Although 2,6-dichloropurine reacted readily with 2,3-dihydrofuran to give V, 2-amino-6-chloropurine (IX) was unreactive under these conditions. In an effort to prepare a 9-(tetrahydro-2-furyl)purine derivative with an amino in the 2-position, V was treated with alcoholic ammonia at 200°. However, the desired 2,6-diamino derivative was not obtained since cleavage of the tetrahydrofuran from the purine moiety occurred under these conditions.

The possibility of utilizing various 2-acetylaminopurine derivatives was then explored. 2-Amino-6-benzyloxypurine (X) was readily prepared from 2-amino-6-chloropurine[14] and sodium in benzyl alcohol after the general method of Montgomery and Balsiger.[15] Acetylation of X with acetic anhydride in refluxing toluene gave 2-acetamido-6-benzyloxypurine (XII) in good yield. Reaction of XII with 2,3-dihydrofuran readily gave 2-acetamido-6-benzyloxy-9-(tetrahydro-2-furyl)purine (XI), which was in turn debenzylated to 2-acetamido-6-hydroxy-9-(tetrahydro-2-furyl)purine (XIII). Finally, XIII was deacetylated with sodium hydroxide to yield 2-amino-6-hydroxy-9-(tetrahydro-2-furyl)purine (XIV). It was readily established that the tetrahydrofuran moiety was at position 9 by a study of the ultraviolet absorption spectrum of XIV which at pH 11 showed λ_{max} of 266 mμ. 9-Methylguanine[16] at pH 11 exhibits λ_{max} at 267 mμ, while 7-methylguanine[7] at pH 11 possesses λ_{max} at 282 mμ.

Reaction Scheme II



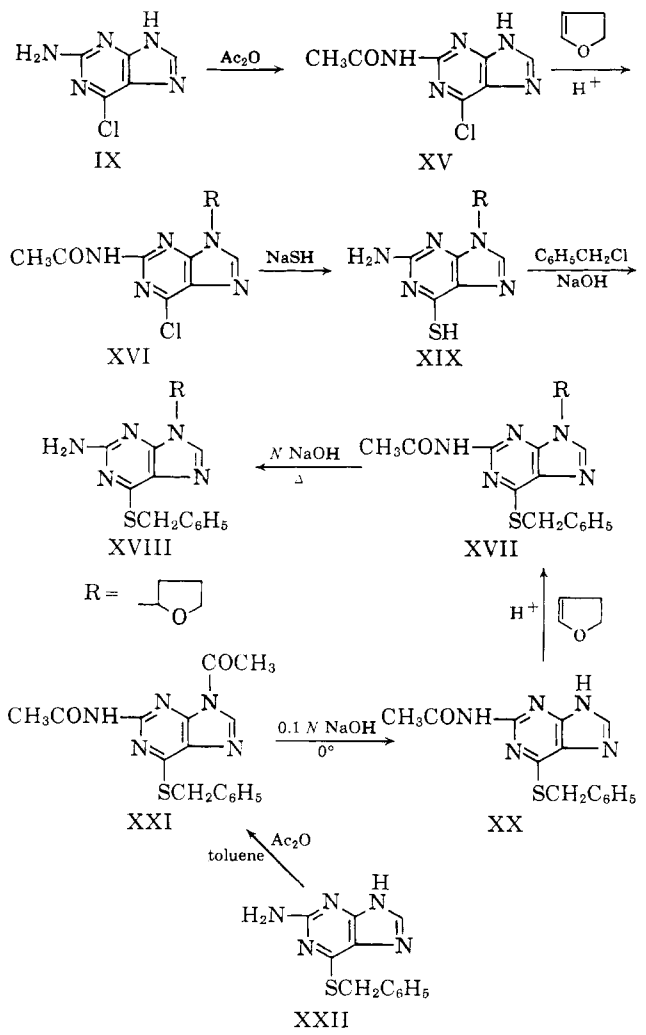
Since some of the most active antitumor agents among purine derivatives have been certain 9-alkyl derivatives of 2-amino-6-purinethiol,[17] it was desirable to obtain 2-amino-9-(tetrahydro-2-furyl)-6-purinethiol (XIX). Because XIV could not be converted directly to XIX with phosphorus pentasulfide and pyridine without loss of the tetrahydrofuran group, other methods had to be devised.

(6) R. K. Robins and H. H. Lin, *J. Am. Chem. Soc.*, **79**, 490 (1957).
(7) R. N. Prasad and R. K. Robins, *ibid.*, **79**, 6401 (1957).
(8) A. G. Beaman and R. K. Robins, *J. Appl. Chem.*, **12**, 432 (1962).
(9) E. Fischer, *Ber.*, **30**, 2400 (1897).
(10) G. Y. Uretskaya, E. I. Rybkina, and G. P. Menshikov, *Zh. Obshch. Khim.*, **30**, 327 (1960).
(11) J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 692 (1938).
(12) A. G. Beaman and R. K. Robins, *J. Org. Chem.*, **28**, 2310 (1963).
(13) W. Bergmann and M. F. Stempien, Jr. *ibid.*, **22**, 1575 (1957).
(14) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, and A. G. Beaman, *J. Am. Chem. Soc.*, **82**, 2633 (1960).
(15) J. A. Montgomery and R. W. Balsiger, *J. Org. Chem.*, **25**, 1573 (1960).
(16) H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).
(17) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).

Selective acetylation of the 2-amino group of 2-amino-6-chloropurine (IX) was investigated under a variety of conditions, and it was found that selective acetylation of the 2-amino group could be obtained with acetic anhydride in N,N-dimethylacetamide to give 2-acetylamino-6-chloropurine (XV). Reaction of XV with 2,3-dihydrofuran gave 2-acetamido-6-chloro-9-(tetrahydro-2-furyl)purine (XVI). Treatment of XVI with 2 *N* sodium hydrosulfide resulted in removal of the acetyl group and simultaneous replacement of the chlorine to yield the desired 2-amino-9-(tetrahydro-2-furyl)-6-purinethiol (XIX). A comparison of the ultraviolet absorption spectra of 2-amino-9-methyl-6-purinethiol[16] and 2-amino-7-methyl-6-purinethiol[7] with that of XIX at pH 11 revealed that the 9-methyl derivative exhibited λ_{max} 252, 268, 317 mμ; the 7-methyl derivative showed λ_{max} 273, 325 mμ, while XIX showed λ_{max} 250, 269, 315 mμ. These data are strong support for the 9-position as the point of attachment of the tetrahydrofuran ring.

Treatment of 2-amino-9-(tetrahydro-2-furyl)-6-purinethiol (XIX) with benzyl chloride in the presence of base readily gave 2-amino-6-benzylthio-9-(tetrahydro-2-furyl)purine (XVIII) which proved to be insoluble in strong base. This was further evidence that the

REACTION SCHEME III



tetrahydrofuryl group was indeed attached to an imidazole nitrogen since a free imidazole hydrogen would have given a base-soluble product. Because of the interesting antitumor properties of XVIII, a second more direct route of synthesis was explored. 2-Amino-6-benzylthiopurine[14] was acetylated with acetic anhydride in toluene to give a diacetyl derivative which has been assigned the structure, 9(7)-acetyl-2-acetamido-6-benzylthiopurine (XXI). Since Montgomery[18] has shown that dilute base at room temperature readily removes an acetyl group attached to position 9(7) of a purine, XXI was selectively deacetylated with 0.1 *N* sodium hydroxide at 0° to give 2-acetamido-6-benzylthiopurine (XX). Treatment of XX with 2,3-dihydrofuran provided an excellent yield of 2-acetamido-6-benzylthio-9-(tetrahydro-2-furyl)purine (XVII). Boiling *N* sodium hydroxide converted XVII to 2-amino-6-benzylthio-9-(tetrahydro-2-furyl)-purine (XVIII), identical in every respect to the product obtained from XIX. 2-Amino-6-benzylthio-9-methylpurine and 2-amino-6-benzylthio-7-methylpurine were prepared by direct benzylation of the requisite 9- and 7-methyl derivatives of 2-amino-6-purinethiol, respectively, in order to compare their ultraviolet absorption spectra with that of XVIII. The ultraviolet absorption spectra revealed that in methanol XVIII possessed λ_{max} at 247 and 314 mμ. 2-Amino-6-benzylthio-9-methylpurine in methanol gave λ_{max} at 247 and 312 mμ, while 2-amino-6-benzylthio-7-methylpurine showed only one absorption maximum in methanol at 325 mμ. This is additional confirmation of the structural assignment given 2-amino-6-benzylthio-9-(tetrahydro-2-furyl)purine (XVIII).

Because of the structural resemblance of the 9-(tetrahydro-2-furyl)purine derivatives to the purine-2′-deoxynucleosides, it was decided to carry the analogy one step further by preparing certain methyltetrahydrofuran derivatives. The ready availability of 2-methyl-2,3-dihydrofuran[19] suggested the use of this intermediate. Thus, 2-methyl-2,3-dihydrofuran and 6-chloropurine (XXIII)[8,20] gave an excellent yield of 6-chloro-9-(tetrahydro-5-methyl-2-furyl)purine (XXIV). Treatment of XXIV with methanolic sodium sulfide led to 9-(tetrahydro-5-methyl-2-furyl)-6-purinethiol (XXV). Ethanolic ammonia at 85° changed XXIV to 6-amino-9-(tetrahydro-5-methyl-2-furyl)-purine (XXVI). Inspection of formula XXIV and the method of synthesis employed reveals that this preparation should consist of two DL-pairs. Several attempts to separate the diastereoisomers of 6-chloro-9-(tetrahydro-5-methyl-2-furyl)purine (XXIV) were unsuccessful. Treatment of XXIV with trimethylamine in benzene gave trimethyl[9-(tetrahydro-5-methyl-2-furyl)-6-purinyl]ammonium chloride (XXVII). This compound is of interest since Horwitz and Vaitkevicius[21] report that 6-purinyltrimethyl-ammonium chloride is active against Ehrlich ascites mouse tumor epidermoid carcinoma DC-5.

Discussion of Antitumor Activity.[22]—The synthesis and antitumor activity of certain 9-substituted purines has been the subject of several previous communications from this laboratory.[6,17,23–25] Noteworthy among

(18) J. A. Montgomery, *J. Am. Chem. Soc.*, **78**, 1928 (1956).
(19) P. Dimroth and H. Pasedach, *Angew. Chem.*, **72**, 865 (1960).
(20) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).
(21) J. P. Horwitz and V. K. Vaitkevicius, *Experientia*, **17**, 552 (1961).
(22) The synthesis of the 9-(tetrahydro-2-furyl)purines discussed will be found either in the present work or in reference 3.
(23) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 1074 (1962).

REACTION SCHEME IV

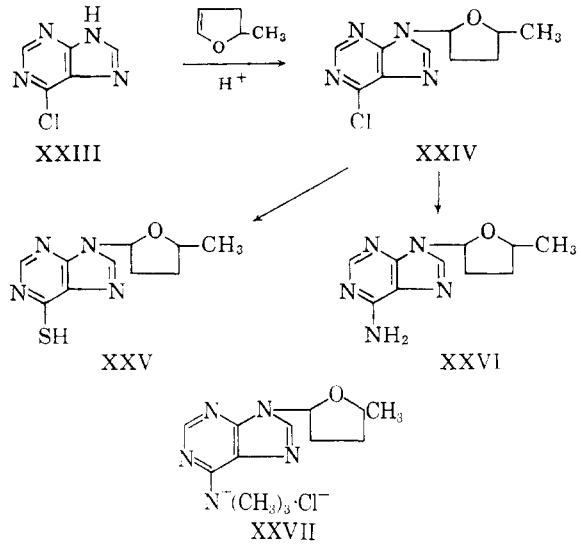
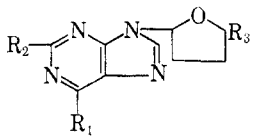


TABLE I

THERAPEUTIC INDICES OF CERTAIN 9-(TETRAHYDRO-2-FURYL)-PURINES AGAINST ADENOCARCINOMA 755



R_1	R_2	R_3	Therapeutic index[a]	Maximum degree of effectiveness T/C at MTD
SH	H	H	200	0.06
SCH_3	H	H	15	.00
NH_2	H	H	10	.00
Cl	H	H	8	.00
p-$SCH_2C_6H_4F$	H	H	8	.00
SH	H	CH_3	8	.00
$SCH_2C_6H_5$	NH_2	H	8	.00
SH	NH_2	H	5	.00
Cl	H	CH_3	4	.00
$^+N(CH_3)_3$	H	H	4	.00
Br	H	H	4	.00

[a] Therapeutic index for Adenocarcinoma 755 is defined by the Cancer Chemotherapy National Service Center as follows: therapeutic index = MTD/MED, where MTD = (maximum tolerated dose) killing not more than 3 out of 10 animals (LD_{30}) with a weight loss between treated and control animals of 5 g. or less. MED (minimum effective dose) = lowest dosage having a T/C of 40% or less.

the compounds described is the activity of 2-amino-9-*n*-propyl-6-purinethiol.[17] Skipper and co-workers[26] have reported the activity of certain 9-alkylpurines against Adenocarcinoma 755 in experimental mice. It has been found recently that certain cycloalkyl groups at position 9 convert 6-chloropurine and 6-purinethiol to compounds which are active against 6-purinethiol-resistant lines of H.Ep. No. 2 in tissue culture studies.[27] Similarly, LePage and Jones[28] have observed that 2-amino-9-methyl-6-purinethiol was inhibitory to a thioguanine-resistant cell line of ascites cells.

Although the mechanism of action of these 9-alkylpurines is presently unknown, it appeared possible that the functional group at position 9 might be taking the place of the carbohydrate moiety and the purine therefore acting at the nucleoside level. This idea receives some support from the work of Kelley, Wheeler, and Montgomery[27] since the 9-cyclohexyl derivative of 6-purinethiol was found to be more inhibitory than the other 9-alkyl-6-purinethiols tested. From this point of view it seemed highly desirable to obtain compounds with the tetrahydrofuran ring at position 9, and, as predicted, the 9-(tetrahydro-2-furyl)purines possess a high degree of antitumor activity in experimental animals. The greatest amount of testing data presently available is against Adenocarcinoma 755. Table I gives the derivatives most active against this tumor. The actual testing data for Adenocarcinoma 755 are given in Table II. Inspection of Table I reveals that 9-(tetrahydro-2-furyl)-6-purinethiol exhibits a therapeutic index of approximately 200 against this tumor. This is compared with a therapeutic index of 30 for 6-mercaptopurine (6-purinethiol)[26] for the same tumor.

A more rigorous therapeutic index employed by Schabel and coworkers[29] is defined as

$$\text{therapeutic index} = \frac{\text{maximum tolerated dose } (LD_{10})}{\text{minimum effective dose } (T/C\ 0.10)}$$

When this definition is employed, 9-(tetrahydro-2-furyl)-6-purinethiol possesses a therapeutic index of 33 as compared with 13 for 6-purinethiol.[29] By this criterion 9-(tetrahydro-2-furyl)-6-purinethiol possesses a therapeutic index against Adenocarcinoma 755 superior to all known purine derivatives with the exception of 6-purinethiol ribonucleoside.[29] The antitumor activity of 9-(tetrahydro-2-furyl)-6-purinethiol is not limited to Adenocarcinoma 755. Table III gives preliminary testing data against Osteogenic Sarcoma HE 10734, L-1210, and Murphy–Sturm Lymphosarcoma. The definite activity of this compound against the two latter tumors is readily apparent. Brockman and co-workers[30,31] have attributed the resistance of neoplasms to 6-purinethiol to be due to a lack of pyrophosphorylase (enzyme activity) which converts 6-purinethiol to its ribonucleotide. Paterson,[32] however, has found that a 6-purinethiol-resistant subline of Ehrlich ascites tumor cells contained the enzyme phosphoribosylpyrophosphate but were resistant to 6-purinethiol because the ability to transport 6-purinethiol through the cell wall has been lost. Thus, it is quite possible that the transport characteristics of the particular form of the drug are extremely important in this instance. It is generally accepted that the nucleotides have great difficulty crossing cell membranes, and it is quite probable that the more lipid-soluble derivatives, such as 9-(tetrahydro-2-furyl)-6-purinethiol, have little trouble in this regard. Kimball and LePage[33] have recently studied the metabolism of radioactive 2-amino-9-*n*-butyl-6-purinethiol in the mouse and noted that the majority of radioactivity was found in the lipid fraction.

(24) A. G. Beaman and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 1067 (1962).
(25) H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).
(26) H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959).
(27) G. G. Kelley, G. P. Wheeler, and J. A. Montgomery, *ibid.*, **22**, 329 (1962).
(28) G. A. LePage and M. Jones, *ibid.*, **21**, 642 (1961).
(29) F. M. Schabel, Jr., J. A. Montgomery, H. E. Skipper, W. R. Laster, Jr., and J. R. Thomson, *ibid.*, **21**, 690 (1961).
(30) R. W. Brockman, C. S. Sparks, M. S. Simpson, and H. E. Skipper, *Biochem. Pharmacol.*, **2**, 77 (1959).
(31) R. W. Brockman, *Cancer Res.*, **20**, 643 (1960).
(32) A. R. P. Paterson, *Can. J. Biochem.*, **40**, 195 (1962).
(33) A. P. Kimball and G. A. LePage, *Cancer. Res.*, **22**, 1301 (1962).

TABLE II

ANTITUMOR ACTIVITY OF 9-(TETRAHYDRO-2-FURYL)PURINES AGAINST ADENOCARCINOMA 755

Compound	Dose, mg./kg.	Survivors	Animal wt. diff. (T − C)	Tumor wt. (test/control)	Per cent (T/C)
6-Chloro-9-(tetrahydro-2-furyl)purine	300	4/10	−4.3	0/1573	Toxic
	150	8/10	−4.0	0/1573	0
	75	9/10	−1.9	0/1106	0
	37.5	10/10	−1.9	15/996	1
	37.5	10/10	−2.7	150/1573	9
	18.75	8/10	−1.7	143/1106	12
	9.37	7/10	−0.5	733/1106	66
	4.68	9/10	−1.1	356/1106	32
	2.34	10/10	−1.4	800/1573	50
6-Methylthio-9-(tetrahydro-2-furyl)purine	150	0/10			Toxic
	75	7/10	−3.0	0/996	0
	37.5	10/10	−2.4	0/996	0
	18.75	9/10	−2.5	17/1033	1
	9.37	9/10	−2.6	0/1033	0
	4.68	9/10	−1.7	122/1033	11
	2.34	8/10	−0.7	479/1033	46
6-Iodo-9-(tetrahydro-2-furyl)purine	200	4/10	−1.8	0/574	Toxic
	100	8/10	−1.1	13/574	2
	50	10/10	−0.8	25/574	4
	25	9/10	−0.3	100/574	17
	12.5	9/10	−0.8	2258/2705	83
	6.2	9/10	−1.5	2058/2705	76
9-(Tetrahydro-2-furyl)-6-purinethiol	200	0/10			Toxic
	100	9/10	−3.5	69/1057	6
	50	9/10	−3.1	64/1057	6
	25	8/10	−2.4	0/1290	0
	12.5	9/10	−1.9	22/1290	1
	6.25	9/10	−2.4	50/1290	3
	3.12	10/10	−4.3	90/1505	5
	3.1	10/10	−2.0	117/1290	9
	1.56	10/10	−3.0	370/1505	24
	.5	9/10	−1.0	277/1057	26
	.39	10/10	−1.0	1210/1505	80
6-Bromo-9-(tetrahydro-2-furyl)purine	200	8/10	−2.6	0/1762	0
	100	9/10	−1.4	44/1762	2
	50	10/10	−1.0	255/1762	14
	25	10/10	−0.6	955/1762	54
6-(*o*-Fluorobenzylthio)-9-(tetrahydro-2-furyl)-purine	200	6/10	−4.2	0/476	Toxic
	100	8/10	−2.7	0/476	0
	50	9/10	−1.3	6/476	1
	25	10/10	−1.7	5/476	1
	25	10/10	−0.5	85/541	15
	12.5	7/10	−0.6	143/541	26
	6.75	9/10	.7	523/541	96
9-(Tetrahydro-2-furyl)-adenine	300	9/10	−6.2	287/1240	23
	250	9/10	−4.0	0/517	0
	150	10/10	−5.3	654/1240	52
	85	10/10	−0.8	5/517	0
	25	9/10	−0.4	11/517	2
	18.7	10/10	.9	1381/1240	111
2-Amino-6-benzylthio-9-(tetrahydro-2-furyl)-purine	60	7/10	−4.3	0/412	0
	30	1/10	−3.1	/412	Toxic
	20	8/10	−3.1	20/676	2
	15	10/10	−2.5	0/412	0
	7.5	10/10	−1.6	11/412	2
	3.7	8/10	−1.3	570/1240	45
	1.8	10/10	−1.1	844/1240	68

Table II (*Continued*)

Compound	Dose, mg./kg.	Survivors	Animal wt. diff. (T − C)	Tumor wt. (test/control)	Per cent (T/C)
Trimethyl[9-(tetrahydro-2-furyl)-6-purinyl]-ammonium chloride	200	1/10	−1.5	/517	Toxic
	150	9/10	−1.2	0/412	0
	100	10/10	−0.6	30/1324	2
	75	10/10	.5	2/412	0
	37.5	10/10	−0.4	21/412	5
	18.7	10/10	1.4	416/412	100
2-Amino-9-(tetrahydro-2-furyl)-6-purinethiol	30	0/10			Toxic
	15	9/10	−1.9	31/1223	2
	7.5	10/10	−2.1	29/1223	2
	3	9/10	−0.7	482/1264	38
	1.5	8/10	−1.2	766/1264	60
9-(Tetrahydro-5-methyl-2-furyl)-6-purinethiol	60	6/10	−4.7	0/1245	Toxic
	30	10/10	−2.9	0/1245	0
	30	9/10	−5.1	0/1155	0
	15	10/10	−2.5	41/1155	3
	7.5	10/10	−3.3	46/1155	3
	3.7	9/10	−1.8	141/1155	12
	1.8	9/10	0.4	999/1155	86
6-Chloro-9-(tetrahydro-5-methyl-2-furyl)purine	240	5/10	−4.1	0/1245	Toxic
	120	10/10	−2.2	8/1245	0
	60	10/10	−2.1	72/1245	5
	30	10/10	−2.3	193/1245	15
	15	10/10	−1.0	734/1155	63

It would be of considerable interest to examine some of the tetrahydrofurylpurines against various purine-resistant neoplasms. There is now ample evidence that suggests these 9-substituted purines do *not* exert their biological activity by merely being dealkylated to the parent purine.[28,33] Kimball and LePage[33] have recently shown that 2-amino-9-*n*-butyl-6-purinethiol is neither dealkylated nor incorporated into nucleic acid in the mouse. The compound 6-amino-9-(tetrahydro-2-furyl)purine is of considerable interest because of its structural relationship to 2′-deoxyadenosine. This compound exhibits a therapeutic index of 10 (Table I) against Adenocarcinoma 755 and also shows some indication of activity against Solid Friend Virus Leukemia (Table III). If the tetrahydrofuran were just a carrier for the adenine molecule, no such antitumor activity would be expected.

It is of interest that in general the 5′-methyltetrahydrofuran derivatives (Tables I and II) are less active than the corresponding derivatives of the unsubstituted tetrahydrofuran nucleus. This could be due to the larger number of isomers involved or other steric factors.

Recently Elion, *et al.*[34] have shown that potentiation of the antitumor action of 6-purinethiol was possible by the concurrent use of another purine antagonist, 4-hydroxypyrazolo(3,4-d)pyrimidine,[35] which decreased the degradation of 6-purinethiol by inhibiting xanthine oxidase. It is quite possible that some of the present 9-(tetrahydro-2-furyl)purines could be used to inhibit enzymatic hydrolysis of other tumor-inhibitory purine nucleosides thus resulting in a similar potentiation. The simultaneous use of more than one active purine derivative has been discussed in a previous paper.[17]

It is interesting to note that often the active 9-(tetrahydro-2-furyl)purines which exhibit antitumor activity are active as the parent purine. This is the case with 6-chloropurine,[36,37] 6-bromopurine,[38] 6-iodopurine,[38] 6-methylthiopurine,[26] and 6-purinyltrimethylammonium chloride.[21] The activity of 9-(tetrahydro-2-furyl)adenine is however a notable exception. The 9-tetrahydrofuryl derivative of thioguanine (2-amino-6-purinethiol) shows good activity in both Adenocarcinoma 755 and Sarcoma 180. This compound is not as toxic as 2-amino-6-purinethiol and shows a much superior inhibition index in Sarcoma 180.

The antitumor activity of the 9-(tetrahydro-2-furyl)purines covers a rather wide spectrum of tumors. Even though limited testing data are presently available, inspection of Table III shows several derivatives significantly active against such experimental mouse tumors as Murphy–Sturm Lymphosarcoma, Dunning Ascites Leukemia, and Spontaneous Mammary Tumors. Of interest is the activity, which awaits further confirmation, of 9-(tetrahydro-2-furyl)adenine against Solid Friend Virus Leukemia (Table III).

Testing procedures, methods, and protocol are adequately described elsewhere[39,40] and were executed under the auspices of the Cancer Chemotherapy National Service Center.

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(38) A. C. Sartorelli, B. A. Booth, and R. K. Robins, *Biochem. Pharmacol.*, **11**, 1017 (1962).

(39) J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *Cancer Res.*, **20**, 734 (1960).

(40) See "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors" (Nov., 1962) available from the Drug Evaluation Branch of the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda 14, Md.

TABLE III

TESTING DATA OF 9-(TETRAHYDRO-2-FURYL)PURINES AGAINST OTHER EXPERIMENTAL MOUSE TUMORS

Compound	Dose, mg./kg.	Survivors	Animal wt. diff. (T − C)	Tumor wt. or Survival, days (test/control)	Per cent (T/C)
Adenocarcinoma of the duodenum					
9-(Tetrahydro-2-furyl)-adenine	143	6/6	3.0	820/864	94
	71.5	6/6	3.0	446/864	51
Murphy–Sturm lymphosarcoma					
9-(Tetrahydro-2-furyl)-6-purinethiol	75	6/6	5.0	1.9/1.9	100
	50	6/6	5.0	3.0/1.9	157
	25	6/6	15.0	3.5/1.9	184
Trimethyl[9-(tetrahydro-2-furyl)-6-purinyl]-ammonium chloride	150	4/6	− 2.0	3.7/1.9	194
	100	6/6	11.0	2.1/1.9	110
	50	6/6	20.0	2.7/1.9	142
	37.5	6/6	0	5.7/4.3	132
	25	6/6	11.0	2.5/1.9	131
Osteogenic sarcoma HE 10734					
9-(Tetrahydro-2-furyl)-6-purinethiol	25	5/6	− 0.3	300/491	61
	12.5	4/6	− 3.4	331/491	67
	6	5/6	− 1.2	332/491	67
Sarcoma 180					
2-Amino-6-benzylthio-9-(tetrahydro-2-furyl)-purine	50	6/6	− 2.3	253/468	54
2-Amino-9-(tetrahydro-2-furyl)-6-purinethiol	30	6/6	− 2.4	132/1002	13
	30	6/6	0.2	329/1101	29
	45	6/6	2.5	293/1593	18
	90	5/6	− 3.1	96/982	9
	20	5/6	− 1.6	416/956	43
Dunning ascites leukemia					
6-Chloro-9-(tetrahydro-2-furyl)purine	250	6/6	−12.0	10.0/7.5	133
	125	6/6	− 6.0	9.0/7.5	120
	62.5	6/6	− 2.0	9.0/7.5	120
Lymphoid leukemia L-1210					
9-(Tetrahydro-2-furyl)-6-purinethiol	144	6/6	− 1.8	13.7/7.8	175
	96	5/6	− 1.7	11.8/7.8	151
	75	6/6	− 2.7	14.8/9.3	159
	50	6/6	− 1.9	12.2/9.3	131
	33.3	6/6	− 1.7	12.5/9.3	134
	22.2	6/6	− 1.4	12.0/9.3	129
2-Amino-9-(tetrahydro-2-furyl)-6-purinethiol	15	6/6	0.6	11.3/9.2	122
Trimethyl[9-(tetrahydro-2-furyl)-6-purinyl]-ammonium chloride	100	6/6	− 1.2	9.2/9.7	94
	50	6/6	0.3	9.0/9.7	92
Solid friend virus leukemia					
9-(Tetrahydro-2-furyl)-adenine	165	3/10	− 2.0	132/327	40
	110	4/10	− 0.6	85/218	39
	110	9/10	− 0.7	222/430	51
	74	10/10	− 0.7	232/327	70
	74	10/10	− 0.4	417/430	96

Spontaneous mammary tumors

Compound	Daily dose, mg./kg.	wk.	Growth index	Wt. change, g.	Deaths	No. inj's.	Av. survival, days
6-Chloro-9-(tetrahydro-2-furyl)-purine	8	3	1.07/1.49	−2.5/−1.0	1/10	11	64.9/33.4
	16	2	1.46/1.45	−1.0/−1.0	0/8	8	42/40.1

Experimental[41]

2-Chloro-6-methylamino-9-(tetrahydro-2-furyl)purine (VII).—To 50 ml. of a 40% aqueous methylamine solution was added 2 g. of 2,6-dichloro-9-(tetrahydro-2-furyl)purine (V). After heating the mixture on a steam bath for 1 hr., a white solid appeared and was filtered to yield 1.7 g. of product, m.p. 183–185°. A small amount was recrystallized from a petroleum ether (b.p. 60–110°) and ethyl acetate mixture for analysis and gave a product melting at 186–188°.

Anal. Calcd. for $C_{10}H_{12}ClN_5O$: C, 47.3; H, 4.73; Cl, 14.2. Found: C, 47.2; H, 5.35; Cl, 14.2.

6-Amino-2-chloro-9-(tetrahydro-2-furyl)purine (VI).—To 45 ml. of methanol, which had previously been saturated with anhydrous ammonia at 0°, was added 5 ml. of concentrated aqueous ammonia, 1 g. of magnesium oxide, and 1 g. of 2,6-dichloro-9-(tetrahydro-2-furyl)purine (V). The mixture was allowed to stand at room temperature for 24 hr. and was then warmed on a steam bath for 1 hr. The solvent was allowed to evaporate at room temperature, and the resulting white solid was extracted with three 50-ml. portions of boiling ethyl acetate. The ethyl

(41) All melting points, taken on a Fisher–Johns apparatus, were determined without correction unless otherwise stated. Much of this work was completed in 1960 and samples and melting point apparatus then used were not available for melting point correction at the date of submission of this manuscript.

acetate was evaporated under reduced pressure to give 0.7 g. of product, m.p. 170–173°. A small sample which was recrystallized from benzene melted at 178–179°.

Anal. Calcd. for $C_9H_{10}ClN_5O$: C, 45.2; H, 4.18; N, 29.3. Found: C, 45.4; 4.18; N, 29.3.

6-Amino-2-methoxy-9-(tetrahydro-2-furyl)purine (VIII).—A solution of 5 ml. of 10% sodium methoxide in methanol and 1.0 g. of 2,6-dichloro-9-(tetrahydro-2-furyl)purine (V) in 50 ml. of absolute methanol was refluxed for 14 hr. The solution was then allowed to cool to room temperature and was carefully neutralized to pH 7 with *N* hydrochloric acid. The excess methanol was evaporated under reduced pressure, and the resulting white solid was filtered, washed with water, and finally triturated with boiling benzene to yield 0.6 g. of product, m.p. 219–221°.

Anal. Calcd. for $C_{10}H_{13}N_5O_2$: C, 51.1; H, 5.53; N, 29.8. Found: C, 51.3; H, 5.80; N, 30.1.

6-Benzyloxypurine (I).—To a solution of 100 ml. of benzyl alcohol, containing 3.0 g. of sodium, was added 10 g. of 6-chloropurine[8,20] in small portions with stirring at 120°. The solution was stirred for 10 hr. at 120–130° and then allowed to cool to room temperature. The cooled solution was neutralized with acetic acid and extracted 15 times with 30-ml. portions of 2 *N* sodium hydroxide. The aqueous extracts were combined, washed with ether, and neutralized with acetic acid. The neutral extract was allowed to stand at 10° for 8 hr. and was then filtered, washed with water, and dried to yield 1.6 g. of product, m.p. 166–168°. Recrystallization from water–methanol raised the melting point to 171–172°.

Anal. Calcd. for $C_{12}H_{10}N_4O$: C, 63.8; H, 4.42; N, 24.8. Found: C, 63.7; H, 4.50; N, 24.9.

6-Benzyloxy-9-(tetrahydro-2-furyl)purine (II).—To 30 ml. of ethyl acetate was added 1.0 g. of 6-benzyloxypurine (I) and 0.1 g. of *p*-toluenesulfonic acid. The mixture was stirred at room temperature, and 1 ml. of 2,3-dihydrofuran was added. The temperature was then increased to 45° and stirring continued for 2 hr. The resulting solution was treated with charcoal, filtered, and allowed to cool to room temperature. The filtrate was washed once with saturated aqueous sodium carbonate and once with water and finally dried over anhydrous sodium sulfate. The excess ethyl acetate was removed under reduced pressure, and the sirupy residue was triturated with petroleum ether (b.p. 30–60°). Upon standing under petroleum ether at 10° for 4 hr., the oil crystallized to yield 0.8 g. of product, m.p. 65–68°. An analytical sample, m.p. 73–74°, was obtained by recrystallization from petroleum ether–ethyl acetate.

Anal. Calcd. for $C_{16}H_{16}N_4O_2$: C, 64.8; H, 5.41; N, 18.9. Found: C, 64.9; H, 5.61; N, 18.8.

6-Hydroxy-9-(tetrahydro-2-furyl)purine (IV). Method 1.—To 50 ml. of absolute ethanol was added 1 g. of 5% palladium-on-charcoal and 2 g. of 6-benzyloxy-9-(tetrahydro-2-furyl)purine (II). The mixture was shaken for 2 hr. at room temperature under 1 atm. of hydrogen. After filtration the ethanol was removed under reduced pressure to yield an oil residue which gradually solidified. The solid was recrystallized from a petroleum ether (b.p. 60–110°)–ethyl acetate to yield 1.1 g. of product, m.p. 176–178°.

Anal. Calcd. for $C_9H_{10}N_4O_2$: C, 52.4; H, 4.86; N, 27.2. Found: C, 52.4; H, 4.82; N, 27.5.

Method 2.—To a stirred solution of 20 ml. of 1.5 *N* sodium hydroxide was added 6.0 g. of trimethyl[9-(tetrahydro-2-furyl)-6-purinyl]ammonium chloride.[8] This solution was heated to 60° for 1 hr., cooled in an ice bath, and neutralized to pH 7 with glacial acetic acid. A white solid separated and was filtered, washed with cold water, and dried at 80° (0.05 mm.) for 1 hr., yield, 2.4 g. (55%). Recrystallization of the material from ethanol gave a product which melted at 172–174°. It possessed an infrared spectrum identical with that of the product prepared by method 1. A mixture melting point of the two preparations was 173–175°. The products possessed the same R_f in 3 different solvent systems. Ultraviolet spectra: pH 1, λ_{max} 249 mμ, ϵ_{max} 7600; pH 11, λ_{max} 253 mμ ϵ_{max} 9680; EtOH, λ_{max} 248 mμ, ϵ_{max} 11,700.

Anal. Calcd. for $C_9H_{10}N_4O$: N, 27.2. Found: N, 27.1.

2-Amino-6-benzyloxypurine (X).—To a solution of 100 ml. of benzyl alcohol, containing 30 g. of sodium, was added 10 g. of 2-amino-6-chloropurine (IX).[14] The reaction mixture was treated as for the preparation of 6-benzyloxypurine (I) to yield 7.9 g. of product, m.p. 197–198°. Recrystallization from water–ethanol raised the melting point to 202–204°.

Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 59.8; H, 4.57; N, 29.1. Found: C, 59.6; H, 4.74; N, 29.2.

2-Acetamido-6-benzyloxypurine (XII).—To 100 ml. of toluene was added 6.0 g. of 2-amino-6-benzyloxypurine (X) and 12 ml. of acetic anhydride. The mixture was refluxed for 4 hr. and then allowed to stand at 10° for 12 hr. The crystalline solid was filtered and washed with benzene to yield 6.1 g. (87%). Recrystallization from methanol gave 5.3 g. of product, m.p. 241–242°.

Anal. Calcd. for $C_{14}H_{13}N_5O_2$: C, 59.3; H, 4.59; N, 24.7. Found: C, 59.4; H, 4.72; N, 24.9.

2-Acetamido-6-hydroxy-9-(tetrahydro-2-furyl)purine (XIII).—To 300 ml. of ethyl acetate, containing 0.1 g. or *p*-toluenesulfonic acid, was added 4.5 g. of 2-acetamido-6-benzyloxypurine (XII). The mixture was stirred at 45–50° for 10 hr., treated with charcoal, and then filtered. The cooled filtrate was washed once with aqueous sodium carbonate (saturated solution) and once with water and finally dried over anhydrous sodium sulfate. The solvent was then evaporated under reduced pressure to obtain 2-acetamido-6-benzyloxy-9-(tetrahydro-2-furyl)purine as a clear oil. The oil was dissolved in 150 ml. of absolute ethanol, and to the resulting solution was added 1 g. of palladium-on-charcoal. The solution was hydrogenated under 80.1 g./m.^{2} pressure for 48 hr. and filtered and the solvent evaporated under reduced pressure. The resulting solid was washed with ethyl acetate and air dried to yield 2.8 g. (decomposed at 259° on a preheated block). A small amount of product was recrystallized from ethyl acetate–methanol to give an analytically pure sample.

Anal. Calcd. for $C_{11}H_{13}N_5O_2$: C, 50.2; H, 4.95; N, 26.8. Found: C, 50.4; H, 5.28; N, 26.5.

2-Amino-6-hydroxy-9-(tetrahydro-2-furyl)purine (XIV). Method 1.—To 50 ml. of *N* sodium hydroxide was added 1 g. of 2-acetamido-6-hydroxy-9-(tetrahydro-2-furyl)purine (XIII). The solution was heated at 80° for 1 hr., cooled, and finally neutralized to pH 7 with acetic acid. The neutral solution was then filtered immediately, and the filtrate was allowed to stand at 10° for 6 hr. The resulting white solid was filtered, washed with cold water, and dried at 80° to yield 0.5 g. (m.p. >300°). Ultraviolet spectra: pH 1, λ_{max} 248 mμ, ϵ_{max} 9,950; pH 11, λ_{max} 266 mμ, ϵ_{max} 10,400; EtOH, λ_{max} 254 mμ, ϵ_{max} 12,800.

Anal. Calcd. for $C_9H_{11}N_5O_2$: C, 48.8; H, 4.98; N, 31.7. Found: C, 48.3; H, 5.18; N, 31.4.

Method 2.—2-Acetamido-6-chloro-9-(tetrahydro-2-furyl)purine (XVI, 200 mg.) was dissolved in 15 ml. of water containing 1.0 g. of sodium hydroxide. This solution was heated and stirred at 80° for 1 hr. The resulting solution was cooled and chromatographed on thin-layer plates (cellulose) against the material obtained by method 1 which had been dissolved in dilute sodium hydroxide. The products showed identical R_f values in three different solvent systems.

2-Acetamido-6-chloropurine[41a] (XV).—To 125 ml. of N,N-dimethylacetamide was added 20 ml. of acetic anhydride, and the solution was stirred and heated to 150°. To this hot solution was added slowly 10.0 g. of 2-amino-6-chloropurine with stirring.[14] All the solid dissolved to give a clear yellow solution. The temperature of the solution was regulated so that it remained at approximately 150°, and after 25 min. at this temperature, the flask was removed from the heat source and allowed to cool. When the solution had cooled to approximately 90°, 100 ml. of ethanol and 25 ml. of water were added. The solution was allowed to cool overnight at room temperature, and the yellow-white solid that formed was filtered, washed with a little acetone, and air dried. The material was recrystallized from a mixture of dimethylacetamide and water to yield 5.5 g. (44.1%) of the desired product, m.p. >300°.

Anal. Calcd. for $C_7H_6ClN_5O$: C, 39.7; H, 2.8; 33.1. Found: C, 39.6; H, 3.0; N, 32.9.

2-Acetamido-6-chloro-9-(tetrahydro-2-furyl)purine (XVI).—To a stirred solution of 250 ml. of ethyl acetate, containing 0.2 g. of *p*-toluenesulfonic acid, was added 10.0 g. of 2-acetamido-6-chloropurine (XV). The solution was heated to 50°, and 10.0 ml. of 2,3-dihydrofuran was added dropwise over a 30-min. period. Stirring and heating were continued for 1 hr. after the addition of the 2,3-dihydrofuran. The solution was cooled to room temperature, and the white solid was filtered and dried *in vacuo*. The yield of dry product was 12.0 g. (90%). Re-

(41a) NOTE ADDED IN PROOF.—The preparation of this compound has recently been reported by a similar method: R. H. Iwamoto, E. M. Acton, and L. Goodman, *Nature*, **198**, 285 (1963).

crystallization of the product from petroleum ether–ethanol gave an analytically pure sample, m.p. 180–181°.

Anal. Calcd. for $C_{11}H_{12}ClN_5O_2$: C, 46.9; H, 4.3; N, 24.8. Found: C, 46.7; H, 4.2; N, 24.8.

2-Amino-9-(tetrahydro-2-furyl)-6-purinethiol (XIX).—To a solution of 140 ml. of freshly prepared 2 *N* sodium hydrosulfide in water and 70 ml. of ethanol was added 5.0 g. of 2-acetamido-6-chloro-9-(tetrahydro-2-furyl)purine (XVI). The solution was refluxed for 1 hr. and then cooled rapidly in an ice bath. The cold solution was carefully neutralized to pH 8 with dilute acetic acid. At about pH 9 a yellow-white solid began to form. This solid was filtered, washed with a little cold water, and dried *in vacuo*. The material was dissolved in base and reprecipitated twice with dilute acetic acid; yield, 3.7 g. (87%). Ultraviolet spectra: pH 1, λ_{max} 344, 259 mμ, ϵ_{max} 20,900, 8150; pH 11, λ_{max} 315, 250 mμ, ϵ_{max} 18,900, 13,300.

Anal. Calcd. for $C_9H_{11}N_5OS \cdot 0.5\,H_2O$: C, 43.9; H, 4.9; N, 28.4; H_2O, 3.7. Found: C, 44.1; H, 4.8; N, 28.4; H_2O, 4.3 (Karl Fischer).

2-Acetamido-9(7)-acetyl-6-benzylthiopurine (XXI).—To 100 ml. of toluene were added 10.0 g. of 2-amino-6-benzylthiopurine[14] and 9.0 ml. of acetic anhydride. The resulting solution was refluxed for 2 hr. and then allowed to cool overnight. The crystalline product was filtered and washed with benzene and then with ether. Recrystallization from ethyl acetate yielded 11.5 g. of white crystals, m.p. 178–180°. Ultraviolet spectra: pH 1, λ_{max} 252, 308 mμ, ϵ_{max} 18,800, 16,000; pH 11, λ_{max} 243, 302 mμ, ϵ_{max} 27,300, 15,400; MeOH, λ_{max} 249, 296 mμ, ϵ_{max} 26,200, 17,400.

Anal. Calcd. for $C_{16}H_{15}N_5O_2S$: C, 56.3; H, 4.4; N, 20.5. Found: C, 56.5; H, 4.8; N, 20.5.

2-Acetamido-6-benzylthiopurine (XX).—To 100 ml. of 0.1 *N* sodium hydroxide at 0° was added with stirring 6.0 g. of 2-acetamido-9(7)-acetyl-6-benzylthiopurine (XXI), and the mixture was stirred at 0–5° for 2 hr. The pH of the solution was adjusted to 7 by the addition of dilute hydrochloric acid, and the material was filtered, washed with water, and recrystallized from ethanol to yield 5.0 g. of white product, m.p. 257–259°. Ultraviolet spectra: pH 1, λ_{max} 252, 310 mμ, ϵ_{max} 15,500, 15,000; pH 11, λ_{max} 242, 302 mμ, ϵ_{max} 25,200, 14,700; MeOH, μ_{max} 249, 296 mμ, ϵ_{max} 26,000, 17,300.

Anal. Calcd. for $C_{14}H_{13}N_5OS$: C, 56.2; H, 4.4; N, 23.4. Found: C, 56.4; H, 4.7; N, 23.4.

2-Acetamido-6-benzylthio-9-(tetrahydro-2-furyl)purine (XVII).—To 150 ml. of ethyl acetate were added 5.0 g. of 2-acetamido-6-benzylthiopurine (XX) and 0.5 g. of *p*-toluenesulfonic acid. 2,3-Dihydrofuran (5.0 g.) was then added with vigorous stirring, and the mixture was heated to 60°. After 30 min. the solution was cooled, washed with saturated aqueous potassium carbonate, and dried over a mixture of anhydrous sodium sulfate and sodium carbonate. The excess ethyl acetate was distilled under reduced pressure, and the sirupy rsidue was recrystallized from acetone–petroleum ether (b.p. 30–60°) to give 4.1 g. of white crystals, m.p. 129–131°. Ultraviolet spectra: pH 1, λ_{max} 253, 306 mμ, ϵ_{max} 18,500, 15,900; pH 11, λ_{max} 250, 297 mμ, ϵ_{max} 21,400, 18,100; EtOH, λ_{max} 252, 297 mμ, ϵ_{max} 25,100, 18,500.

Anal. Calcd. for $C_8H_{19}N_5O_2S$: C, 58.5; H, 5.2; N, 19.0. Found: C, 58.6; H, 5.3; N, 18.6.

2-Amino-6-benzylthio-9-(tetrahydro-2-furyl)purine (XVIII). Method 1.—To 75 ml. of *N* sodium hydroxide was added 5.0 g. of 2-acetamido-6-benzylthio-9-(tetrahydro-2-furyl)purine (XVII). The mixture was refluxed with vigorous stirring for 2 hr. and then cooled in an ice bath and filtered. The product was washed thoroughly with water, and recrystallization from benzene–petroleum ether (b.p. 60–110°) gave 3.6g. of colorless crystals, m.p, 168–170°. Ultraviolet spectra: pH 1, λ_{max} 2.7, 321mμ, ϵ_{max} 11,500, 17,300; pH 11, λ_{max} 313mμ, ϵ_{max} 14,400 EtOH, λ_{max} 247, 313 mμ, ϵ_{max} 17,300, 15,400.

Anal. Calcd. for $C_{16}H_{17}N_5OS$: C, 58.7; H, 5.2; N, 21.4. Found: C, 58.5; H, 5.3; N, 21.3.

Method 2.—To 15 ml. of *N* sodium hydroxide was added 200 mg. of 2-amino-9-(tetrahydro-2-furyl)-6-purinethiol (XIX). This solution was heated to 40°, and any undissolved material was filtered. The solution was cooled to room temperature, and while stirring 1 ml. of benzyl chloride was added dropwise over a 15-min. period. The solution was stirred for an additional hour, during which time a white solid formed. The solid was filtered, washed with a small amount of cold water, and dried *in vacuo*. The material was recrystallized from benzene–petroleum ether to give a product melting at 168–170°. The product showed no depression in melting point when mixed with an authentic sample of 2-amino-6-benzylthio-9-(tetrahydro-2-furyl)purine. The infrared spectrum was identical with that of the product prepared by method 1, and chromatograms in two solvent systems also showed this product to be identical with that prepared by method 1.

6-Chloro-9-(tetrahydro-5-methyl-2-furyl)purine (XXIV).—To 300 ml. of ethyl acetate was added 10.0 g. of 6-chloropurine and 0.5 g. of *p*-toluenesulfonic acid. This mixture was heated to 50° while stirring, and 10.0 g. of 2-methyl-2,3-dihydrofuran[42] was added dropwise over a period of 1 hr. The ethyl acetate solution was filtered to remove traces of unreacted 6-chloropurine. The solution was washed with aqueous saturated sodium bicarbonate solution and then with cold water and finally dried over sodium sulfate. The ethyl acetate was removed *in vacuo*, and the yellow oil that remained was poured into 250 ml. of boiling petroleum ether (30–75°) which after cooling yielded 10.4 g. of white needles, m.p. 73–74°.

Anal. Calcd. for $C_{10}H_{11}ClN_4O$: C, 50.3; H, 4.6; N, 23.5. Found: C, 50.3; H, 4.5; N, 23.3.

9-(Tetrahydro-5-methyl-2-furyl)-6-purinethiol (XXV).—To a solution of 80 ml. of sodium hydrosulfide in methanol (prepared by dissolving 5 g. of sodium in 100 ml. of methanol and saturating this solution at 0° with hydrogen sulfide) was added a solution of 2.4 g. of 6-chloro-9-(tetrahydro-5-methyl-2-furyl)purine (XXIV) dissolved in 40 ml. of methanol. This solution was heated for 1 hr. on a steam bath, cooled to 0°, and carefully neutralized to pH 7 with glacial acetic acid. The white solid that separated was filtered and washed with cold water. The solid was covered with 50 ml. of water, and a small amount of sodium hydroxide was added until complete solution was obtained. The solution was then cooled to 5° and neutralized with glacial acetic acid. The product obtained (2.0 g.) melted at 223–225°.

Anal. Calcd. for $C_{10}H_{12}N_4OS$: C, 50.8; H, 5.1; N, 23.7. Found: C, 50.4; H, 5.0; N, 24.0.

6-Amino-9-(tetrahydro-5-methyl-2-furyl)purine (XXVI).—6-Chloro-9-(tetrahydro-5-methyl-2-furyl)purine (XXIV, 2.0 g.) was dissolved in 150 ml. of ethanolic ammonia (saturated at 0°) and the solution heated to 85° for 5 hr. in a stainless steel bomb. The resulting brown solution was diluted with 150 ml. of ethyl acetate and filtered to remove the ammonium chloride. Potassium hydroxide pellets (0.5 g.) were added and the solution evaporated to dryness *in vacuo*. The remaining brown oil was extracted with 70 ml. of boiling petroleum ether (60–110°) and then with 100 ml. of boiling benzene. The benzene extract was treated with Norit and evaporated to a total volume of 25 ml. Petroleum ether (30–60°) was added to the benzene solution until a precipitate formed. This solid was filtered and recrystallized from benzene–petroleum ether (30–60°) to yield 1.6 g. of white crystals, m.p. 127–128°.

Anal. Calcd. for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.9; N, 32.0. Found: C, 54.8; H, 5.7; N, 31.9.

Trimethyl[9-(tetrahydro-5-methyl-2-furyl)-6-purinyl]ammonium chloride (XXVII).—To a solution of 5.0 g. of 6-chloro-9-(tetrahydro-5-methyl-2-furyl)purine (XXIV) in 50 ml. of anhydrous benzene was added a solution of 3.0 g. of anhydrous trimethylamine in 50 ml. of benzene. Immediately a white solid began to separate from the solution. The solution was stirred for 3 hr. at room temperature, after which time the white solid that had formed was filtered, washed with benzene, and dried in a vacuum desiccator to yield 5.3 g. of product, m.p. 90–91°.

Anal. Calcd. for $C_{13}H_{20}ClN_5O$: C, 52.4; H, 6.7; N, 23.4. Found: C, 52.1; H, 7.2; N, 23.0.

2,6-Dichloro-9-(tetrahydro-2-furyl)purine (V).—To 150 ml. of ethyl acetate were added 10.0 g. of 2.6-dichloropurine[8] and 0.5 g. of *p*-toluenesulfonic acid. 2,3-Dihydrofuran[43] (5.6 g.) was then added dropwise with stirring. The mixture was stirred at room temperature for 15 min. and then slowly heated to 50° at which point all solid material had dissolved. The solution was then cooled to room temperature, washed with saturated aqueous sodium carbonate, and dried over a mixture of anhydrous magnesium sulfate and sodium carbonate. The excess ethyl acetate was removed *in vacuo* and the liquid residue allowed to crystallize. Recrystallization from a mixture of petroleum ether (b.p. 60–110°) and ethanol gave 8.3 g. of colorless crystals, m.p. 110–111°. Ultraviolet spectrum: EtOH, λ_{max} 274 mμ, ϵ_{max} 9000.

(42) See ref. 19. The authors wish to thank Badische Anilin- & Soda-Fabrik A. G. for a generous sample of 2-methyl-2,3-dihydrofuran.

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Anal. Calcd. for $C_9H_8Cl_2N_4O$: C, 41.7; H, 3.1; N, 21.6. Found: C, 41.8; H, 3.3; N, 21.4.

2-Amino-6-benzylthio-9-methylpurine.—To a stirred solution of 150 ml. of concentrated aqueous ammonia, containing 7.0 g. of 2-amino-9-methyl-6-purinethiol,[16] was added 20 ml. of *p*-dioxane and 5.0 g. of benzyl chloride. This mixture was stirred and heated (40°) for 1 hr. during which time the product precipitated from the solution and was filtered, washed with water, and dried. The crude product was recrystallized from ethyl acetate–petroleum ether (60–110°) to give 3.6 g. Recrystallization from benzene provided a sample with a melting point of 131–133°. Ultraviolet spectra: pH 1, λ_{max} 321 mμ, ϵ_{max} 11,700; pH 11, λ_{max} 313 mμ, ϵ_{max} 12,500; MeOH, λ_{max} 313 mμ, ϵ_{max} 12,500.

Anal. Calcd. for $C_{13}H_{13}N_5S$: C, 57.7; H, 4.8; N, 24.2. Found: C, 58.2; H, 5.1; N, 23.9.

2-Amino-6-benzylthio-7-methylpurine.—2-Amino-7-methyl-6-purinethiol[7] (150 mg.) was suspended in 5 ml. of water, and enough *N* sodium hydroxide was added to the suspension to effect solution. Benzyl chloride (3 drops) was added to this solution, and the solution was shaken at room temperature for 15 min. and cooled in an ice bath. The product was filtered and washed with a small volume of water (yield 100 mg.). Recrystallization was accomplished from benzene to give a product, m.p. 192–193°. Ultraviolet spectra: pH 1, λ_{max} 322, 280 mμ, ϵ_{max} 14,900, 10,300; pH 11, λ_{max} 323 mμ, ϵ_{max} 11,000; MeOH, λ_{max} 326 mμ, ϵ_{max} 11,000.

Anal. Calcd. for $C_{13}H_{13}N_5S$: C, 57.7; H, 4.8; N, 24.2. Found: C, 57.4; H, 4.7; N, 24.7.

Solvents Used in Chromatography.—The following three solvent systems were employed throughout this work: (1) 5% ammonium bicarbonate in water; (2) water saturated with *n*-butyl alcohol; (3) 5% disodium hydrogen phosphate in water saturated with isoamyl alcohol

The Biological and Physical Properties of the Azaindoles

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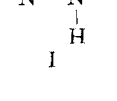
Received February 6, 1963

Incorporation of an isoelectronic nitrogen atom in place of a methine group in the indole molecule increases the basic strength and reduces the lipid–water distribution ratio. Depending on the position of the doubly bonded nitrogen atom, the azaindoles exert pharmacological effects on smooth muscle and the central nervous system that either mimic or oppose the actions of indole.

Pharmacological interest in the azaindoles stems from the possibility that they may serve as the parent nucleus of active or antagonistic analogs of naturally occurring indole derivatives such as the endogenously important serotonin, melatonin, and tryptophan[2-4] and the potent pharmacological agents such as psilocybin and lysergic acid diethylamide. Furthermore, it seemed likely that unsubstituted azaindoles would have pharmacological properties because (unsubstituted) indole has marked pharmacologic effects; it depresses smooth muscle and produces convulsions originating in the spinal cord and subthalamic areas of the brain.[5-9] Of the six isomeric monoazaindoles, only one, benzimidazole (3-azaindole), has been studied *in vivo*: it was found to produce a transient flaccid paralysis owing to interneuronal depression.[10-13] Because virtually nothing is known of the biological actions of other mono- and polyazaindoles, their acute pharmacological effects in mice and their *in vitro* effects on smooth muscle have been determined in the present study. The determination of the lipid–water solubility ratio of the nonionized form, and the calculation of the degree of ionization at body pH, are also included in this paper because these physical properties are known to have a marked influence on the activity of centrally active agents.[14,15]

Experimental

Materials.—Commercial preparations of indole, 2-azaindole (1H-indazole), 3-azaindole (benzimidazole), 7-azaindole (I) (1H-pyrrolo[2,3-b]pyridine), and 2,3-diazaindole (benztriazole) were purified by recrystallization from hot water followed by high vacuum sublimation. All other mono- and polyazaindoles used in this work were synthesized in the Department of Medical Chemistry of the Australian National University, Canberra and their purification has been described.[16-19] The compounds include: 4-azaindole (1H-pyrrolo[3,2-b]pyridine); 5-azaindole (1H-pyrrolo[3,2-c]pyridine); 6-azaindole (1H-pyrrolo[2,3-c]pyridine); 3,4-diazaindole (1H-imidazo[4,5-b]pyridine); 3,5-diazaindole (1H-imidazo[4,5-c]pyridine); and purine (7H-imidazo[4,5-d]pyrimidine).



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(1) Supported by Career Development Award GM-K3-7415 and research grant B-2508 from the National Institutes of Health, USPHS. It is a pleasure to acknowledge the assistance of Mr. R. Frieman, Mr. M. Laueson, and Mr. M. Abdel-Rahman in some of the experiments.

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