Isomerization of 2,4,6-Tris-(1-aziridinyl)-*s*-triazine (X).—A mixture of 50 ml. of acetone, 500 mg. of NaI and 1.0 g. of X[4] was kept at room temperature for 2 hours. During this time 2,3,6,7,10,11-hexahydrotrisimidazo[1,2-a;1',2'-c;1'',2''-e]-*s*-triazine (XI) settled out. The filtered product weighed 881 mg. and melted at 320°. A mixed melting point with a sample of XI prepared by the method of Schaefer[4] showed no depression of melting point. Infrared spectra of the isomerized product and the authentic sample were identical.

Isomerization of 1-Aziridinecarboxanilide (XII).—A mixture of 2.015 g. of XII,[5] 1.02 g. of NaI and 7 ml. of acetone was refluxed 16 hours and then placed in an ice-bath. In a short time the 1-phenyl-2-imidazolidinone crystallized. It was filtered and weighed (1.7 g.). The crude product melted at 155–160°. Some of the imidazolidinone was recrystallized from water to give product melting at 161–163°. A mixed melting point determination and comparison of infrared spectra with an authentic sample of 1-phenyl-2-imidazolidinone prepared by the alkaline solvolysis of N-phenyl-N'-2-chloroethylurea[5] unequivocally identified the isomerized product as the imidazolidinone.

Dimerization of XII.—A mixture of 2.010 g. of XII, 100 ml. of acetone and 300 mg. of NaI was refluxed for 40–50 hours during which time N,N'-bisphenylcarbamylpiperazine (XIII) gradually precipitated. The solution was filtered while hot to give 450 mg. of XIII which decomposed at 308° with some sublimation. An authentic sample of XIII was prepared by treating phenyl isocyanate with piperazine.[24] Comparison of infrared spectra and a mixed melting point determination of the reaction product with the true sample established the structure. The literature value[24] for the decomposition point is 305–310° with accompanying sublimation. Longer reaction times (96 hours) did not increase the yield of XII.

The following experiment was so designed to give evidence that some 2-anilino-2-oxazoline also forms. A mixture of 1.01 g. of 1-aziridinecarboxanilide, 615 mg. of oven-dried NaI and 40 ml. of acetone (previously dried over K_2CO_3 and distilled) was refluxed for 52 hours. The solvent was evaporated, the residue washed with about 4 ml. of water and filtered. The residue weighed 1.01 g. and was washed with 20–25 ml. of ether. The ether extract was evaporated to give 160 mg. of material melting from 85–110°. A recrystallization from heptane gave crystals melting at 108–114° with slight sintering at 98°. The infrared spectrum was identical with that of a sample of 2-anilino-2-oxazoline prepared by the method of Gabriel and Stelzner[6] who reported a melting point of 119–120°. Two strong absorption peaks occur at 9.5 and 16.28 μ for the oxazoline. The 1-phenyl-2-imidazolidinone does not absorb in this region. The imidazolidinone strongly absorbs at 15 and 17.2 μ. The 2-anilino-2-oxazoline obtained from the reaction of 1-aziridinecarboxanilide with NaI did not show any absorption peaks at 15 and 17.2 μ. A Perkin–Elmer Infracord Spectrophotometer Model 137–KBr was used for these measurements.

A control run was made by refluxing 1.13 g. of 1-aziridinecarboxanilide and 25 ml. of acetone for 40 hours. The solvent was evaporated and the residue weighed. The recovered residue weighed (1.07 g.), melted at 78–82° and had an infrared spectrum identical with the starting compound.

Acknowledgment.—E. M. J. wishes to thank the National Science Foundation for an Undergraduate Research Participation Grant.

(24) F. Wrede and E. Panik, *Z. physiol. Chem.*, **131**, 49 (1923).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZONA; THE RESEARCH LABORATORIES, PARKE, DAVIS & CO., DETROIT, MICHIGAN, AND THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY, PRINCETON, NEW JERSEY]

Purine Nucleosides. I. The Synthesis of Certain 6-Substituted-9-(tetrahydro-2-pyranyl)-purines as Models of Purine Deoxynucleosides[1]

BY ROLAND K. ROBINS, ERIK F. GODEFROI, EDWARD C. TAYLOR, LELAND R. LEWIS AND ALVIN JACKSON

RECEIVED OCTOBER 13, 1960

2,3-Dihydro-4H-pyran and various 2-substituted 2,3-dihydro-4H-pyrans have been shown to react with certain 6-substituted purines in the presence of a catalytic amount of acid to give the corresponding 6-substituted-9-(tetrahydro-2-pyranyl)-purines. This reaction provides a new synthetic route to the preparation of valuable models of certain purine deoxynucleosides which possess significant antitumor activity. The tetrahydropyran molecule greatly increases the solubility of purine derivatives in organic solvents and provides a useful blocking group which can be readily used to prevent undesirable side reactions due to the presence of the imidazole hydrogen. Acid hydrolysis regenerates the purine.

There is considerable evidence[2–5] that 6-purinethiol (6-mercaptopurine) exerts its antitumor activity in the form of its nucleoside or nucleotide. It would appear that resistance to this drug is due to the loss of the capacity of the cell to form the nucleotide of 6-purinethiol.[2–5] Since intact purine nucleotides are unable to penetrate mammalian cells[6] without extensive dephosphorylation, it would seem worthwhile to synthesize a number of nucleoside and nucleotide analogs which would be less polar and which might as a consequence possess the characteristics necessary for passage through the cancer cell wall. These model nucleosides should resemble the naturally occurring purine nucleosides sufficiently to be accepted by the appropriate enzymes of the cancer cell. Such compounds might well exhibit antitumor activity against the strains of tumor which have become resistant to the usual simple purine antagonists.[7]

The synthesis of a number of 9-arylpurines[8–10] has revealed that although the substitution of a phenyl ring in position 9 resulted in purines of

(1) Supported in part by Contract SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute of the National Institutes of Health.

(2) A. R. P. Paterson, *Proc. Am. Assoc. Cancer Res.*, **3**, 50 (1959).

(3) J. D. Davidson, *Cancer Res.*, **20**, 225 (1960).

(4) S. Tomizawa and L. Aronow, *J. of Pharm. and Exp. Therapeutics*, **128**, 107 (1960).

(5) J. S. Salser, D. J. Hutchinson and M. E. Balis, *J. Biol. Chem.*, **235**, 429 (1960).

(6) P. M. Roll, H. Weinfeld, E. Carroll and G. B. Brown, *ibid.*, **220**, 439 (1956).

(7) For a review of purines exhibiting antitumor properties, see H. E. Skipper, J. A. Montgomery, J. R. Thomson and F. M. Schabel, Jr., *Cancer Research*, **19**, 425 (1959).

(8) H. C. Koppel and R. K. Robins, *J. Am. Chem. Soc.*, **80**, 2751 (1958).

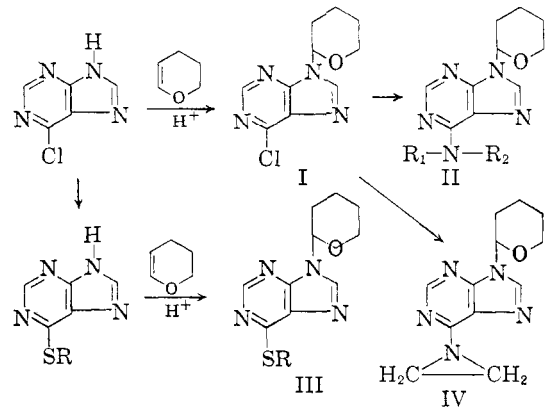
(9) H. C. Koppel, D. E. O'Brien and R. K. Robins, *ibid.*, **81**, 3046 (1959).

(10) S. M. Greenberg, L. O. Ross and R. K. Robins, *J. Org. Chem.*, **24**, 1314 (1959).

lowered polarity, these compounds exhibited little antitumor activity as compared to the simple purines.[7]

It therefore seemed advisable to prepare purine derivatives which would, in addition to possessing low polarity, also more closely resemble the purine nucleosides and thus possess the steric requirements necessary for acceptance by the purine nucleoside enzyme systems. Schaeffer and Weimar[11] have reported the synthesis of certain 6-chloro- and 6-mercaptocycloaliphatic purines which have retained some of the tumor inhibiting properties of the parent purines.[7] In the present study, purine derivatives possessing a tetrahydropyranyl ring system at position 9 were selected for synthesis. The problem involved in the introduction of the tetrahydropyranyl ring when considered in the light of existing methods was not hopeful. Thus, available methods for the introduction of a substituent at position 9 in the purine ring can be summarized as follows: (1) synthesis of the requisite 5-amino-4-substituted-aminopyrimidine followed by subsequent ring closure,[9,10,12] (2) cyclization of the requisite 5-substituted ureido- or thioureidopyrimidine,[8,13,14] (3) cyclization of an appropriate 1-substituted 5-amino-4-cyano-,[15] 4-amido-[16,17] or 4-carbethoxy-[16] imidazole and (4) direct alkylation of a purine derivative by an alkyl halide.[11,18,19,20] None of these methods seemed applicable due to the unavailability of the necessary tetrahydropyran derivatives and probable instability of the requisite pyrimidine or imidazole intermediates. The reaction of certain 6-substituted purines directly with 2,3-dihydro-4H-pyran, however, readily provided the desired compounds.

For example, 6-chloropurine[21] and 2,3-dihydro-4H-pyran[22] in the presence of a catalytic amount of *p*-toluenesulfonic acid gave 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) in good yield. This represents a new method of introducing a substituent at position 9 of the purine moiety and is currently being further investigated with a view to the possible utilization of appropriate glycols for a new route to the synthesis of purine nucleosides. Theoretically, the reaction of 6-chloropurine and 2,3-dihydro-4H-pyran could give rise to either 7- or 9-substitution or a mixture of both isomers. Careful examination of the reaction mixture revealed the presence of only one major purine derivative which possessed a sharp melting point and exhibited only one spot when chromatogrammed in several different inert solvents. The tetrahydro-2-pyranyl group was assigned to position 9 by comparison of the ultraviolet absorption spectrum of I in ethanol with that of 6-chloro-9-methylpurine[23] and 6-chloro-7-methylpurine.[24] Both I and 6-chloro-9-methylpurine in ethanol exhibit a maximum at 265 mμ while 6-chloro-7-methylpurine possesses a maximum at 271 mμ. Further structure proof was obtained by conversion of I to 6-dimethylamino - 9 - (tetrahydro - 2 - pyranyl)-purine (II, R_1, R_2 = CH_3). Baker, Schaub and



Joseph[25] have shown that 9-methyl-6-dimethylaminopurine exhibits absorption maxima at 270 and 277 mμ at *p*H 1 and 14, respectively, and 7-methyl-6-dimethylaminopurine under identical conditions exhibits absorption maxima at 290 and 295 mμ at *p*H 1 and 14, respectively. Compound II, R_1, R_2 = CH_3, exhibits maxima at 270 mμ at *p*H 1 and 277 mμ at *p*H 14. This leads to the definite assignment of the tetrahydropyranyl group of II, R_1, R_2 = CH_3, to position 9. Similar reasoning was employed by Baker, Schaub and Joseph[25] in the assignment of the structure of puromycin. Since 6-dimethylamino-9-(tetrahydro-2-pyranyl)-purine was prepared from I, the structure of I must be 6-chloro-9-(tetrahydro-2-pyranyl)-purine.

Treatment of I with alcoholic ammonia at 100° gave 6-amino-9-(tetrahydro-2-pyranyl)-purine (II, R_1, R_2 = H), a nucleoside model of deoxyadenosine.

A study of Table II reveals that a similar comparison of the ultraviolet absorption spectra of 6-amino-9-(tetrahydro-2-pyranyl)-purine (II, R_1, R_2 = H), 9-methyladenine and 7-methyladenine results in similar assignment of the tetrahydropyranyl group to position 9. Gulland and Holiday[26] correctly assigned the structure of adenosine by means of a similar comparison of its ultraviolet absorption spectra with the spectra exhibited by 7- and 9-methyladenine.

A further study of the reaction of 2,3-dihydro-4H-pyran with various 6-substituted purines re-

(11) H. J. Schaeffer and R. D. Weimar, Jr., *J. Am. Chem. Soc.*, **81**, 197 (1959).

(12) R. K. Robins and H. H. Lin, *ibid.*, **79**, 490 (1957).

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

(14) F. F. Blicke and R. L. Schaaf, *J. Am. Chem. Soc.*, **78**, 5857 (1956).

(15) G. Shaw and D. N. Butler, *J. Chem. Soc.*, 4040 (1959).

(16) A. H. Cook, J. D. Downer and Sir Ian Heilbron, *ibid.*, 1069 (1949).

(17) A. H. Cook and E. Smith, *ibid.*, 2329 (1949).

(18) J. A. Montgomery and C. Temple, *J. Am. Chem. Soc.*, **83**, 630 (1961).

(19) This is essentially the procedure employed in treatment of the silver or mercury salt of a purine with a halosugar in the synthesis of purine nucleosides. See J. J. Fox, *Record of Chemical Progress*, **19**, 173 (1958), for review of this method.

(20) J. M. Gulland and T. F. Macrae, *J. Chem. Soc.*, 662 (1933).

(21) A. Bendlich, P. J. Russell, Jr., and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

(22) 2,3-Dihydro-4H-pyran is commercially available from the Quaker Oats Co.

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

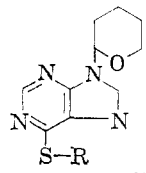
(24) R. N. Prasad and R. K. Robins, *ibid.*, **79**, 6401 (1957).

(25) B. R. Baker, R. E. Schaub and J. P. Joseph, *J. Org. Chem.*, **19**, 638 (1954).

(26) J. M. Gulland and E. R. Holiday, *J. Chem. Soc.*, 765 (1936).

TABLE I

SOME 6-ALKYLTHIO-9-(TETRAHYDRO-2-PYRANYL)-PURINES



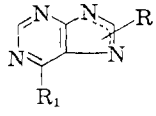
R	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Yield, %	Recrystallization solvent
$CH_2C_6H_4Cl$-*p*	94–95	56.7	56.6	4.9	4.7	15.5	15.4	68	Petroleum ether
$CH_2C_6H_4Cl$-*o*	84–86	56.7	57.0	4.9	4.7	15.5	15.3	50	Petroleum ether
$CH_2C_6H_4F$-*o*	100	59.3	59.1	4.9	5.0	16.3	16.3	72	Petroleum ether
$CH_2C_6H_4F$-*m*	89–90	59.3	59.2	4.9	5.0	16.3	16.2	78	Petroleum ether
$CH_2C_6H_5$	104	62.6	62.5	5.5	5.6	17.2	17.1	85	Benzene–heptane
$CH_2C_6H_4F$-*p*	106–107	59.3	59.2	5.0	5.0	16.3	16.5	87	Benzene–heptane
i-C_4H_9	101–102	57.4	57.1	6.8	6.4	19.1	19.0	63	Petroleum ether
n-C_4H_9	107–108	57.4	57.4	6.8	6.7	19.1	18.9	57	Petroleum ether
n-C_3H_7	94	56.1	56.1	6.5	6.4	20.1	20.0	66	Petroleum ether
CH_3	93–94	52.9	53.0	5.6	5.7	22.4	22.4	78	Petroleum ether

vealed that the reaction was successful in the case of 6-iodopurine, 6-methoxypurine and 6-methylthiopurine. The reaction conditions employed for the preparation of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) were unsuccessful for the attachment of the tetrahydropyranyl group directly to adenine, hypoxanthine, purine or 6-purinethiol. The compound 9-(tetrahydro-2-pyranyl)-6-purinethiol (III, R = H), however, was readily prepared from I and methanolic sodium hydrosulfide.

The reaction of 2,3-dihydro-4H-pyran and 6-methylthiopurine[27] in the presence of a catalytic amount of *p*-toluenesulfonic acid proceeded so well that the reaction was extended to the synthesis of a number of 6-alkylthio-9-(tetrahydro-2-pyranyl)-purines (III) by treatment of the requisite alkylthiopurine[28] with 2,3-dihydro-4H-pyran. These derivatives are listed in Table I. Examination of Table II will reveal that a comparison of the ultra-

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA[a] OF CERTAIN 9-TETRA-HYDRO-2-PYRANYL-6-SUBSTITUTED PURINES AND RELATED 7- AND 9-METHYLPURINES



R_1	7-Methyl[24] λmax, mμ	7-Methyl[24] ε	9-Methyl[23] λmax, mμ	9-Methyl[23] ε	9-Tetrahydro-2-pyranyl λmax, mμ	9-Tetrahydro-2-pyranyl ε
NH_2	272	9,500	262	12,500	261	14,000
Cl	271	7,300	265	9,100	265	8,800
SCH_3	293	14,000	284	17,800	284	19,000
$SCH_2C_6H_5$	295	16,100	286	20,000	286	21,200
SH	330	17,900	324	21,900	324	21,600
HN–CH_3	278	12,400	268	15,300	267	15,100
S-*n*-C_4H_9	294	13,300			285	16,100
S-*n*-C_3H_7	294	14,600			285	17,000
$SCH_2C_6H_4F$-*p*	295	14,500			286	19,000

[a] All spectra in this table were determined in absolute ethanol.

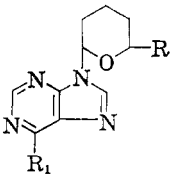
violet absorption data for III, R = CH_3 or R = $CH_2C_6H_5$, requires the assignment of the tetrahydropyranyl group once again to position 9. 6-Benzylthio-9-methylpurine and 6-benzylthio-7-methylpurine were prepared from 9-methyl-6-purinethiol and 7-methyl-6-purinethiol specifically for this study by treatment of the corresponding purinethiol with benzyl chloride. The synthesis of 7-methyl-6-*n*-butylthiopurine and 7-methyl-6-*p*-fluorobenzylthiopurine revealed that these derivatives exhibited ultraviolet absorption spectra very similar to those exhibited by 7-methyl-6-methylthiopurine and 7-methyl-6-benzylthiopurine, respectively, and quite unlike the spectra of 6-*n*-butylthio-9-(tetrahydro-2-pyranyl)-purine (III, R = *n*-C_4H_9) and 6-*p*-fluorobenzylthio-9-(tetrahydro-2-pyranyl)-purine [III, R = $CH_2C_6H_4F(p)$] (see Table II). The useful protecting action of the tetrahydropyranyl group is strikingly demonstrated by the successful synthesis of 6-aziridinyl-9-(tetrahydro-2-pyranyl)-purine (IV) from ethylenimine and I. This reaction fails with 6-chloropurine. Compound IV is of interest since it can be considered an alkylating agent structurally related to deoxyadenosine. Other substituted amino derivatives prepared from I were 6-methylamino-9-(tetrahydro-2-pyranyl)-purine (II, R_1 = H, R_2 = CH_3) and 6-furfurylamino-9-(tetrahydro-2-pyranyl)-purine (II, R_1 = H, R_2 = furfuryl). Since most of the 9-(tetrahydro-2-pyranyl)-purines here described possess significant antitumor activity against adenocarcinoma 755 in mice, this study was extended to the preparation of several 9-(tetrahydro-2-pyranyl)-purines substituted in position 6 of the pyranyl ring. 2-Ethoxy- and 2-methoxy-2,3-dihydro-4H-pyran[29] were found to react readily with 6-chloropurine in the expected manner to give the corresponding 9-(6-alkoxytetrahydro-2-pyranyl)-6-chloropurine. It should be noted that since 2,3-dihydro-4H-pyran reacts with 6-chloropurine to give a racemic mixture, the reaction of DL-2-alkoxy-2,3-dihydro-4H-pyran and 6-chloropurine presumably gives rise to a mixture of 4 isomeric optically active products. No effort was made at this time to resolve any of these reaction products. Several additional 9-(6-alkoxytetrahydro-2-pyranyl)-6-substituted purines were pre-

(27) G. B. Elion, E. Burgi and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

(28) H. C. Koppel, D. E. O'Brien and R. K. Robins, *J. Org. Chem.*, **24**, 259 (1959).

(29) Kindly supplied by the Shell Development Co., Emeryville, California.

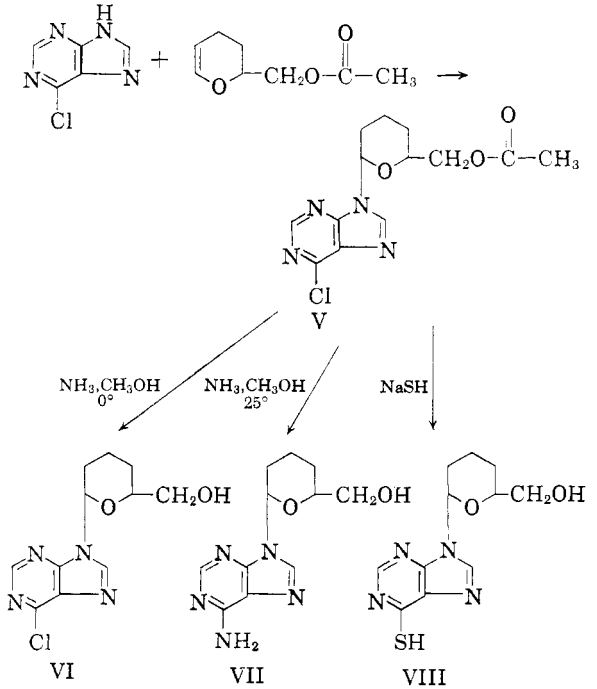
TABLE III
9-(6-ALKOXYTETRAHYDRO-2-PYRANYL)-6-SUBSTITUTED PURINES



R	R_1	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Yield, %	Recrystallization solvent	λmax, ethanol mμ	ε
OCH_3	Cl	116–117	49.1	49.1	4.8	5.0	20.8	20.7	35	Petroleum ether	265	9,900
OCH_3	SH	221 dec.	49.7	50.1	5.3	5.4	21.0	20.8	72	Dimethylformamide and water	325	22,600
OCH_3	SCH_3	112–113	51.6	51.5	5.4	5.9	20.1	19.8	40	Petroleum ether	284	19,500
OC_2H_5	Cl	107–109	51.1	51.4	5.1	5.2	19.8	19.7	63	Petroleum ether	265	9,400
OC_2H_5	I	165–166 dec.	38.5	38.8	4.0	4.2	14.9	14.8	66	Benzene	276	11,200
OC_2H_5	SH	225–226 dec.	51.5	51.1	5.4	5.4	20.1	20.4	49	Dimethylformamide and water	325	21,300
OC_2H_5	$N(CH_3)_2$	97–97.5	57.5	57.2	7.2	7.3	24.1	24.5	67	*n*-Heptane	275	18,600

pared by essentially the same procedure employed in the reactions utilizing 2,3-dihydro-4H-pyran. These compounds are listed in Table III.

When 2,3-dihydropyran-2-methanol[30] was employed with 6-chloropurine, no reaction took place, presumably due to the preferential self-condensation of 2-hydroxymethyl-2,3-dihydro-4H-pyran with its own hydroxyl group in the presence of acid.



However, when this hydroxyl group was first acetylated, the reaction proceeded in the normal fashion. Thus, 2-acetoxymethyl-2,3-dihydro-4H-pyran[30] and 6-chloropurine gave 6-(6-chloro-9-purinyl)-tetrahydropyran-2-methanol acetate V in excellent yield. This reaction is of considerable interest since it suggests that certain acetylated unsaturated sugars (glycals) might be successfully employed in a new synthesis of purine deoxynucleosides. Efforts in this direction are presently under investigation. Deacetylation of V occurred at 0° with methanolic ammonia to give 6-(6-chloro-9-purinyl)-tetrahydropyran-2-methanol (VI) while the same reagent at 25° resulted in simultaneous replacement of the 6-chloro atom to give VII an analog of adenosine. The utility of V was further demonstrated by the fact that sodium hydrosulfide provided 6-(6-thio-9-purinyl)-tetrahydropyran-2-methanol (VIII) directly since replacement of the chloro group and deacetylation proceeded simultaneously under the reaction conditions employed.

A detailed report of the antitumor activity of these compounds will be reported elsewhere.

Acknowledgments.—The authors wish to thank Dr. Gerhard Usbeck for the preparation of 6-benzylthio-9-methylpurine needed for this study. The authors also gratefully acknowledge the assistance of Howard Schneider relative to this work.

Experimental[31]

Preparation of 6-Chloro-9-(tetrahydro-2-pyranyl)-purine (I).—To 600 ml. of anhydrous ethyl acetate warmed to 50° were added 60 g. of 6-chloropurine[21] and 1 g. of *p*-toluenesulfonic acid. The mixture was vigorously stirred and 40 ml. of 2,3-dihydropyran[22] added dropwise over a 30-min. period, maintaining the reaction temperature between 55–60°. The solution was stirred for an additional hour during which time it was allowed to cool to room temperature. Concentrated aqueous amonia (35 ml.) was added and the solution stirred for 5 min. It was then extracted twice with 200 ml. of water. The ethyl acetate layer was dried over anhydrous sodium sulfate and finally removed under reduced pressure utilizing a steam-bath as a source of heat. The remaining syrupy residue crystallized on standing. The crude product was extracted with 5 × 400 ml. portions of boiling petroleum ether (65–85°). The cooled petroleum ether yielded 61.2 g. of white needles, m.p. 67–69°. A second recrystallization from petroleum ether (65–85°) raised the melting point to 69–71°. An additional 8.1 g. of product was obtained by concentration of the petroleum ether extracts.

Anal. Calcd. for $C_{10}H_{11}ClN_4O$: C, 50.3; H, 4.6; N, 23.5. Found: C, 50.3; H, 4.8; N, 23.1.

6-Amino-9-(tetrahydro-2-pyranyl)-purine (II, R_1, R_2 = H).—Ten grams of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) was dissolved in 250 ml. of absolute ethanol which had been previously saturated with ammonia at 0°. The solution was placed in a stainless steel bomb and heated at 100° for 2.5 hr. To the clear resulting solution was added 3 g. of

(30) R. Zelinski, A. Verbiscar and H. J. Eichel, *J. Org. Chem.*, **23**, 184 (1958).

(31) All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected.

solid potassium hydroxide which gradually dissolved. The potassium chloride which appeared was filtered from the solution and the ethanol removed under reduced pressure. The residual solid was extracted with 3 × 200 ml. of boiling ethyl acetate; the combined extract was concentrated to 200 ml. and cooled. The colorless needles which appeared were filtered and washed with a little cold ethyl acetate to yield 3.5 g., m.p. 186–188°. Further concentration of the ethyl acetate gave an additional 1.4 g. of product. Recrystallization from ethyl acetate did not alter the melting point.

Anal. Calcd. for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.9; N, 32.0. Found: C, 54.5; H, 6.3; N, 31.9.

6-Methylthio-9-(tetrahydro-2-pyranyl)-purine (III, R = CH_3).—To 300 ml. of ethyl acetate was added 30 g. of anhydrous 6-methylthiopurine[27] and 0.5 g. of *p*-toluenesulfonic acid. The solution was heated to 55–60° with stirring while 40 ml. of 2,3-dihydropyran was added over a 30-min. period. Heating was then discontinued and the clear solution stirred for 30 min. more. The ethyl acetate solution was then shaken with 100 ml. of a saturated aqueous solution of sodium carbonate, followed by washing with 2 × 200 ml. of water. The solution was dried over anhydrous potassium carbonate. Distillation of the ethyl acetate under reduced pressure left a residual syrup which crystallized to a solid mass overnight. Recrystallization of the solid from petroleum ether (65–85°) gave 37.1 g. of product, m.p. 90–93°. A second recrystallization from the same solvent raised the melting point to 93–94°.

Anal. Calcd. for $C_{11}H_{14}N_4OS$: C, 52.9; H, 5.6; N, 22.4. Found: C, 53.0; H, 5.7; N, 22.4.

The 6-alkylthio-9-(tetrahydro-2-pyranyl)-purines listed in Table I were prepared in a similar manner from 2,3-dihydro-4H-pyran and the appropriate 6-alkylthiopurine.[28]

9-(Tetrahydro-2-pyranyl)-6-purinethiol.—Seven grams of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) was dissolved in 150 ml. of absolute methanol. To this solution was added 200 ml. of a solution of sodium hydrosulfide in methanol prepared as follows: (To 200 ml. of absolute methanol was added 9 g. of sodium, and the cooled solution was saturated with hydrogen sulfide). The solution was gently refluxed for 20 min., and the precipitate of sodium chloride which appeared was filtered from the hot solution. To the hot filtrate was carefully added glacial acetic acid until *p*H 7 was reached. The cooled solution was filtered and the white product washed with water and dried to yield 5.3 g. of product uncontaminated by 6-purinethiol as evidenced by chromatographic analysis in several different solvents. 9-(Tetrahydro-2-pyranyl)-6-purinethiol was recrystallized from N,N-dimethylformamide for analysis to give needles which gradually decomposed above 225°.

Anal. Calcd. for $C_{10}H_{12}N_4SO$: C, 50.9; H, 5.1; N, 23.7. Found: C, 51.2; H, 5.0; N, 23.5.

6-(1-Aziridinyl)-9-(tetrahydro-2-pyranyl)-purine (IV).—To 200 ml. of benzene, 20 ml. of triethylamine and 10 ml. of ethylenimine was added 10 g. of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I). The solution was stirred at 45–50° for 2 hr. and then boiled gently on the steam-bath for 5 min. and allowed to cool overnight. The solution was filtered and the excess solvent removed under reduced pressure using a water-bath (60°) as a source of heat. The colorless liquid which remained was extracted with 2 × 125 ml. of boiling petroleum ether (80–110°). The solution was cooled and the product filtered to give 5.3 g., m.p. 80–85°. Recrystallization from petroleum ether raised the melting point to 88–89°.

Anal. Calcd. for $C_{12}H_{15}N_5O$: C, 58.8; H, 6.1; N, 28.6. Found: C, 58.8; H, 5.7; N, 28.8.

6-Dimethylamino-9-(tetrahydro-2-pyranyl)-purine (II, R_1,R_2 = CH_3).—Seven grams of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) and 150 ml. of 25% aqueous dimethylamine were heated for 8 hr. on the steam-bath until the volume was reduced to 75 ml. The hot solution consisted of two phases which were separated while hot in a small separatory funnel. The heavier oily layer was then added to 150 ml. of boiling benzene and the volume reduced to 75 ml. to remove the last traces of water as an azeotrope. An equal volume of petroleum ether was then added to the benzene and the solution allowed to cool. A crude yield of 4.3 g. of light-tan crystals was obtained, m.p. 124–126°. Recrystallization from *n*-heptane (using charcoal) gave colorless crystals, m.p. 127–128°.

Anal. Calcd. for $C_{12}H_{17}N_5O$: C, 58.3; H, 6.9; N, 28.4. Found: C, 58.8; H, 6.7; N, 28.4.

6-Methylamino-9-(tetrahydro-2-pyranyl)-purine (II, R_1 = H, R_2 = CH_3).—Seven grams of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) was added to 120 ml. of 40% aqueous methylamine and the solution heated to dryness on the steam-bath. The residue was dissolved in boiling methanol which deposited 3.4 g. of crystals on cooling, m.p. 164–166°. Recrystallization from benzene raised the melting point to 167–169°.

Anal. Calcd. for $C_{11}H_{15}N_5O$: C, 56.6; H, 6.5; N, 30.0. Found: C, 56.7; H, 6.5; N, 30.4.

6-Iodo-9-(tetrahydro-2-pyranyl)-purine.—To 300 ml. of ethyl acetate (anhydrous), heated to 60°, was added 53 g. of 6-iodopurine,[32] 1 g. of *p*-toluenesulfonic acid and 40 ml. of 2,3-dihydropyran, dropwise, over a 30-min. period. The product was isolated and purified in a manner similar to that employed for the preparation of I to yield (after recrystallization from petroleum ether) 62 g. of colorless needles, m.p. 98–100°. The product in ethanol exhibited λ_{max} 275 mμ, $\epsilon_{12,900}$.

Anal. Calcd. for $C_{10}H_{11}N_4OI$: C, 36.4; H, 3.3; N, 16.9. Found: C, 36.8; H, 3.4; N, 17.1.

6-Furfurylamino-9-(tetrahydro-2-pyranyl)-purine.—To 3 ml. of furfurylamine was added 1 g. of 6-chloro-9-(tetrahydro-2-pyranyl)-purine (I) and the solution heated to 90° for 25 min. The solution was cooled and 40 ml. of ether added. The furfurylamine hydrochloride was filtered, and the filtrate was concentrated to give a residual oil which slowly crystallized. Isooctane (20 ml.) was added and the product filtered and washed with a little light petroleum ether to give 1.3 g. After recrystallization from absolute ethanol, the colorless cubes melted at 138–139°.

Anal. Calcd. for $C_{15}H_{17}N_5O_2$: C, 60.2; H, 5.7; N, 23.3. Found: C, 60.3; H, 5.8; N, 23.4.

6-*n*-Butylthio-7-methylpurine.—7-Methyl-6-purinethiol[24] (1.7 g.) was dissolved in 50 ml. of water containing 0.7 g. of potassium hydroxide. Then 1.8 g. of 1-iodobutane was added and the solution stirred vigorously at room temperature for 2 hr. The solid was filtered and recrystallized from water to give 0.9 g. of white needles, m.p. 118–119°.

Anal. Calcd. for $C_{10}H_{14}N_4S$: C, 54.2; H, 6.3; N, 25.3. Found: C, 54.5; H, 6.4; N, 25.4.

6-Benzylthio-7-methylpurine.—7-Methyl-6-purinethiol[24] (1.7 g.) was added to 50 ml. of water containing 0.7 g. of potassium hydroxide. To this solution was added 1.4 g. of α-chlorotoluene. The solution was vigorously stirred for 2 hr., and the reaction mixture was cooled and filtered. The solid was recrystallized from water to give 2.1 g. of white crystals, m.p. 155–157°.

Anal. Calcd. for $C_{13}H_{12}N_4S$: C, 60.9; H, 4.7; N, 21.8. Found: C, 60.5; H, 4.5; N, 21.6.

6-(*p*-Fluorobenzylthio)-7-methylpurine.—This compound was prepared from 7-methyl-6-purinethiol and *p*-fluorobenzyl chloride in a manner similar to that described for the preparation of 6-benzylthio-7-methylpurine to give a yield of 85% of white crystals. The crude product was recrystallized from benzene–heptane to give white needles, m.p. 163–165°.

Anal. Calcd. for $C_{13}H_{11}N_4SF$: C, 56.9; H, 4.0; N, 20.6. Found: C, 56.8; H, 3.5; N, 20.6.

6-Benzylthio-9-methylpurine.—To 150 ml. of water and 10 g. of potassium hydroxide was added 7.2 g. of 9-methyl-6-purinethiol.[23] The solution was heated to 80°, and a solution of 45.6 g. of α-chlorotoluene in 20 ml. of *p*-dioxane was added dropwise over a 30-min. period. The solution was stirred at 75° for 1 hr. and then cooled overnight in the refrigerator. The product was filtered and recrystallized twice from ethanol to give 4.2 g., m.p. 121–123°.

Anal. Calcd. for $C_{13}H_{12}N_4S$: C, 60.9; H, 4.7; N, 21.8. Found: C, 60.9; H, 4.6; N, 21.4.

6-(6-Chloro-9-purinyl)-tetrahydropyran-2-methanol Acetate (V).—To 600 ml. of anhydrous ethyl acetate heated to 70° were added 30 g. of 6-chloro-purine and 2 g. of *p*-toluenesulfonic acid. The mixture was stirred vigorously, and 60 g. of 2-acetoxymethyl-2,3-dihydro-4H-pyran[30] was added

(32) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 3508 (1956).

dropwise over a 30-min. period, during which time the temperature was maintained at 70–75°. The heating was then discontinued and the solution stirred for 1 hr. at room temperature. The resulting solution was then washed once with a saturated solution of sodium carbonate, twice with water and dried over anhydrous sodium sulfate. The ethyl acetate was then removed under reduced pressure, and the residue was suspended in 200 ml. of anhydrous ethyl ether and filtered. Recrystallization of the solid from an ethyl acetate-*n*-heptane mixture gave 46 g. of colorless crystals, m.p. 139–140°.

Anal. Calcd. for $C_{13}H_{15}ClN_4O_3$: C, 50.2; H, 4.8; N, 18.1. Found: C, 49.9; H, 4.8; N, 18.0.

6-(6-Chloro-9-purinyl)-tetrahydropyran-2-methanol (VI). —Ten grams of 6-(6-chloro-9-purinyl)-tetrahydropyran-2-methanol acetate (V) was dissolved in 150 ml. of methanol which had previously been saturated with ammonia at 0°. The resulting solution was stirred vigorously at 0° for 8 hr. then placed overnight in the refrigerator. The methanol and excess ammonia were removed under reduced pressure. To the syrupy residue was added 100 ml. of petroleum ether and the product allowed to crystallize. Recrystallization from benzene–petroleum ether (b.p. 60–110°) gave 4.5 g. of white product, m.p. 139–140°.

Anal. Calcd. for $C_{11}H_{14}ClN_4O_2$: C, 49.2; H, 4.8; N, 20.8. Found: C, 49.4; H, 5.1; N, 20.6.

6-(6-Thio-9-purinyl)-tetrahydropyran-2-methanol (VIII) —Eight grams of 6-(6-chloro-9-purinyl)-tetrahydropyran-2-methanol acetate (V) was dissolved in 125 ml. of absolute methanol. To this solution was added 250 ml. of a solution of sodium hydrosulfide in methanol (prepared as in the preparation of 9-(tetrahydro-2-pyranyl)-6-purinethiol). The solution was heated on the steam-bath for 30 min. and filtered. The filtrate was carefully neutralized to *p*H 7 with glacial acetic acid. The solution was then cooled and the product collected by filtration. Recrystallization of the crude product from a dimethylformamide–water mixture gave 6.5 g. of pale-yellow crystals which decomposed, without melting, > 200°.

Anal. Calcd. for $C_{11}H_{14}N_4SO_2$: C, 49.7; H, 5.3; N, 21.0. Found: C, 49.6; H, 5.4; N, 20.9.

6-(6-Amino-9-purinyl)-tetrahydropyran-2-methanol (VII). —6-(6-Chloro-9-purinyl)-tetrahydropyran-2-methanol acetate (V) (25 g.) was added to 250 ml. of ethanol, which had previously been saturated with anhydrous ammonia at 0°, and the resulting solution was allowed to stand at room temperature for 48 hr. The solution was then evaporated to dryness and the residue suspended in 200 ml. of chloroform and filtered. Recrystallization of the solid from absolute ethanol gave 18.7 g. of colorless leaf-shaped crystals, m.p. 200–201°.

Anal. Calcd. for $C_{11}H_{15}N_5O_2$: C, 53.1; H, 6.0; N, 28.2. Found: C, 53.1; H, 6.1; N, 28.4.

COMMUNICATIONS TO THE EDITOR

EVIDENCE FOR NITROGEN MIGRATION IN THE BENZILIC ACID REARRANGEMENT OF ALLOXAN AND DERIVATIVES

Sir:

The benzilic acid rearrangement of alloxan (I) to alloxanic acid[1] (II) has been the subject of a recent[2] kinetic study, on the basis of which it was suggested that three different anions could be formed, any one of which could conceivably be a rearrangement intermediate. These results led to the proposal[2] that not only a carbon–carbon shift but also a nitrogen–carbon shift was possible during the rearrangement I → II. Although both possibilities had been suggested before,[1b,3] the work of Kwart and Sarasohn[2] appears to be the first presumptive evidence that a nitrogen-carbon shift during a benzilic acid rearrangement is possible. Tracer studies with carbon-14 now have been carried out which show unambiguously that the nitrogen shift takes place to the exclusion of the carbon shift during rearrangement of alloxan and several of its derivatives under widely differing conditions of *p*H. Alloxan [Ia, $R^1 = R^2 = H$] labeled in the 5-position was prepared by oxidation with chromic anhydride of the barbituric acid obtained from urea and methylene-labeled malonic ester[4]; it

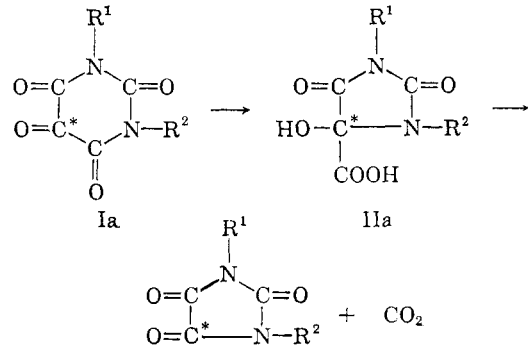


CHART I

was then subjected to rearrangement (a) at *p*H 7–10 in potassium hydroxide solution; (b) at *p*H 13 in sodium hydroxide solution; (c) at *p*H 9.4 in sodium hydroxide–sodium borate buffered solution; (d) at *p*H 7.2–7.5 in sodium hydroxide–sodium phosphate buffered solution and (e) at *p*H *ca.* 1 in nitric acid. In the experiments performed under alkaline conditions the alloxanic acid (IIa) was oxidized to parabanic acid IIIa with nitric acid solution, whereas in the acid-catalyzed rearrangement, parabanic acid [IIIa] was formed directly. Radioactivity assay of IIIa and Ia (or its barbituric acid precursor) or IIa demonstrated that the samples of IIIa possessed 98.5–102.6% of the original radioactivity (see Chart I). Several derivatives of alloxan labeled in the 4-positions[5]

(1) (a) F. Wöhler and J. V. Liebig, *Ann.*, **26**, 241 (1838); A. Schlieper, *ibid.*, **263**, 55 (1845); (b) H. Biltz, M. Heyn and M. Bergius, *ibid.*, **413**, 68 (1916); (c) G. M. Richardson and R. K. Cannon, *Biochem. J.*, **23**, 68 (1928); (d) J. W. Patterson, A. Lazarow and S. Levey, *J. Biol. Chem.*, **177**, 187 (1949).

(2) H. Kwart and I. Sarasohn, *J. Am. Chem. Soc.*, **83**, 909 (1961).

(3) (a) S. Selman and J. F. Eastham, *Quarterly Reviews*, **14**, 234 (1960); (b) F. R. Fisher and R. A. Day, *J. Am. Chem. Soc.*, **77**, 4895 (1955).

(4) According to the procedure of A. V. Holmgren and W. Wenner, *Organic Syntheses*, **32**, 6 (1952), John Wiley and Sons, New York, N. Y.

(5) The synthetic route employed in the synthesis of N-methylalloxan and of N-phenylalloxan starts with the appropriate monosubstituted urea and cyanoacetic-*carboxyl*-C^{14} acid to yield $RNHCONHC^*OCH_2CN$, which undergoes ring closure in the presence of