H_2O, and Raney nickel W2 (from *ca.* 1.5 g of Ni–Al alloy) was added following which hydrogenation was carried out as in method a. After removal of catalyst 1 equiv of HCl was added and the solution was extracted ($CHCl_3$) to remove traces of starting material. The aqueous layer was evaporated *in vacuo* to small volume and the amine hydrochloride precipitated with acetone. To remove the isopropylidene group a solution of 0.5 g of this product in 25 ml of HCl (pH 2) was heated for 1 hr at 70–80°. After removal of most of the H_2O the hydrochloride was precipitated with acetone. Reprecipitation yielded 300 mg (69%) from the above hydrochloride), mp 217° *Anal.* ($C_{10}H_{15}N_5O_4 \cdot HCl \cdot H_2O$) C, H, N.

2',3'-O-Isopropylidene-3,5'-cycloinosine (9).—A suspension of **5** (1 g) in 5 ml of concentrated NH_4OH was stirred for 24 hr. The crystalline product (0.59 g, 80.5%) was filtered and washed (H_2O); upon chromatography in systems A, B, and C it showed only one spot. Recrystallization (EtOH) gave white plates: mp 266° dec; at pH 4, λ_{max} 253 mμ (ε 8430); at pH 11, λ_{max} 256 mμ (ε 8430). *Anal.* ($C_{13}H_{14}N_4O_4 \cdot H_2O$) C, H, N. Holmes and Robins[10] also obtained a monohydrate.

5'-O-(*p*-Nitrobenzenesulfonyl)-2',3'-O-isopropylideneinosine (11).—*p*-Nitrobenzenesulfonyl chloride (850 mg) was added to a suspension of dry 2',3'-O-isopropylideneinosine (**10**) (800 mg) in 6 ml of anhydrous pyridine cooled in ice. The mixture was shaken until the 2',3'-O-isopropylideneinosine dissolved (15 min) and set aside for 10 hr at 2°. Pyridine was removed *in vacuo* and the residual gum was dissolved at 2° with 80 ml of $CHCl_3$ and the solution was extracted at 2° with 0.01 *N* HCl (two 30-ml portions). The $CHCl_3$ solution was dried (Na_2SO_4) and concentrated *in vacuo* to *ca.* 20 ml. C_6H_6 (15 ml) and Me_2CO (15 ml) were added and the volume was reduced *in vacuo* to 10–15 ml to give 0.77 g (60%) of white crystals, mp 181° dec. The product gave only one uv-absorbing spot on paper chromatography (solvents A and C) and tlc on silica gel in $CHCl_3$–CH_3OH (93:7) (R_f 0.45). In H_2O (pH 4) or CH_3OH, λ_{max} was 248 mμ. At pH 4, ε was 15,900; this high value is presumably due to contribution from the *p*-nitrobenzenesulfonoxy group, since methyl *p*-nitrobenzenesulfonate in EtOH has λ_{max} 250 mμ (ε 11,200).[13] Crystallization (Me_2CO) gave needles, mp 181°. *Anal.* ($C_{18}H_{18}N_5O_8S$) C, H, N.

(13) J. P. Phillips and F. C. Nachod, Ed., "Organic Electronic Spectral Data," Vol. IV, Interscience Publishers, Inc., New York, N. Y., 1958, p 115.

Acylations of Some 2-Amino-6-halo- and 2-Amino-6-alkylthiopurines[1]

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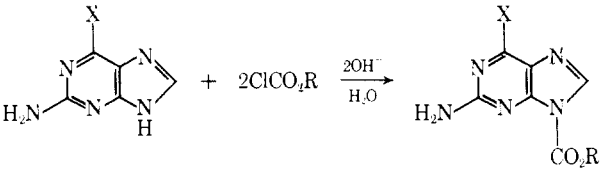
Received July 29, 1968

The present work is a continuation of a study of acylation of purines[2,3] which involves the preparation of derivatives of possible value in cancer chemotherapy. Included in the current work are carboxylate derivatives of 2-aminopurine-6-thione and of 2-amino-6-chloropurine, both of which in the free state have tumor-inhibitory properties.[4,5]

The 2-amino group in purines is known to react with both aliphatic and aromatic anhydrides[6-9] to yield the corresponding acylamido derivatives and, when the work-up does not prevent isolation, the purine ring is also acylated.[6,7,9] This is similar to the reactivity of the 6-amino group of adenine.[10-12] However, the 6-amino group of adenine is unreactive toward ethyl chloroformate in aqueous base; acylation occurs on the purine nucleus.[3]

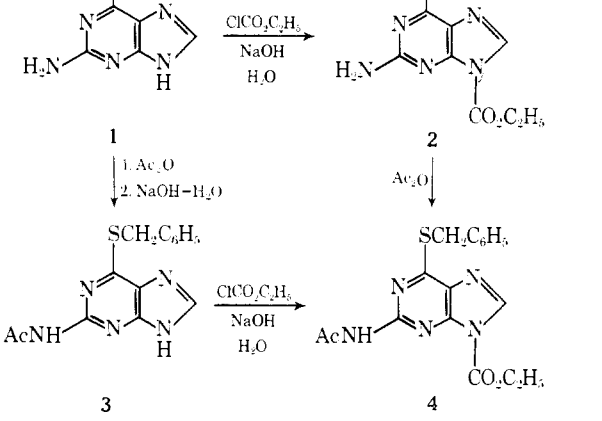
The current results show that the 2-amino group also is unreactive toward alkyl chloroformates under Schotten–Baumann conditions. A number of 2-amino-6-halo- and 2-amino-6-alkylthiopurines yielded monoacyl derivatives (Table I) when treated with excess ethyl or methyl chloroformate and the site of acylation was shown to be the imidazole ring.



2, X = $SCH_2C_6H_5$; R = C_2H_5
8, X = SCH_3; R = C_2H_5
10, X = $SCH_2C_6H_5$; R = CH_3
12, X = Br; R = CH_3
5, X = Cl; R = C_2H_5
9, X = SCH_3; R = CH_3
11, X = Br; R = C_2H_5
13, X = Cl; R = CH_3

The 2-amino group was excluded as the site of acylation by an independent synthesis based on the known 2-acetamido-6-benzylthiopurine[6] (**3**), since the reaction of ethyl chloroformate with **3** gave the same compound **4** as did the reaction of Ac_2O with ethyl 2-amino-6-benzylthiopurine-9-carboxylate (**2**) (Scheme I).

SCHEME I



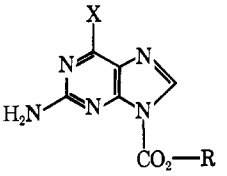
The preparation of carbethoxy derivatives of 2-aminopurine-6-thione and 2-amino-6-selenopurine was accomplished by the action of thiourea and selenourea on **5**. Although these reagents have been widely used with 6-chloropurine and its derivatives,[13-16] little use of this reagent has been made with 2-amino-6-chloropurine.[17]

In the current work the reaction of thiourea with **5** in refluxing EtOH gave 2-(2-amino-9-carbethoxypurin-

(1) (a) This investigation was supported by the Public Health Service Research Grant No. CA-03477 from the National Cancer Institute. (b) Abstracts from the Ph.D. thesis of C. E. M. (1968), University of Delaware.
(2) E. Dyer and H. Bender, *J. Med. Chem.*, **7**, 10 (1964).
(3) E. Dyer, J. M. Reitz, and R. E. Farris, Jr., *ibid.*, **6**, 289 (1963).
(4) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.*, **18**, 445 (1958).
(5) R. K. Robins, *J. Med. Chem.*, **7**, 186 (1964).
(6) W. A. Bowles, F. H. Schneider, L. R. Lewis, and R. K. Robins, *ibid.*, **6**, 471 (1963).
(7) R. H. Iwamoto, E. M. Acton, and L. Goodman, *ibid.*, **6**, 684 (1963).
(8) J. Davoll and B. A. Lowy, *J. Am. Chem. Soc.*, **73**, 1650 (1951).
(9) B. R. Baker, K. Hewson, H. J. Thomas, and J. A. Johnson, *J. Org. Chem.*, **22**, 954 (1957).
(10) A. Kossel, *Z. Physiol. Chem.*, **12**, 246 (1888).
(11) L. Birkofer, *Chem. Ber.*, **76B**, 769 (1943).
(12) A. H. Schein, *J. Med. Pharm. Chem.*, **5**, 302 (1962).
(13) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).
(14) E. F. Melnerney and E. J. Kupchik, *J. Med. Chem.*, **10**, 741 (1967).
(15) H. G. Mautner, *J. Am. Chem. Soc.*, **78**, 5292 (1956).
(16) H. J. Schaeffer and E. Odin, *J. Med. Chem.*, **9**, 576 (1966).
(17) A recent exception is the work of J. F. Gerster, B. C. Hinshaw, R. K. Robins, and L. B. Townsend, *J. Org. Chem.*, **33**, 1070 (1968).

TABLE I

CARBOXYLATE DERIVATIVES OF 2-AMINO-6-HALO- AND 2-AMINO-6-ALKYLTHIOPURINES

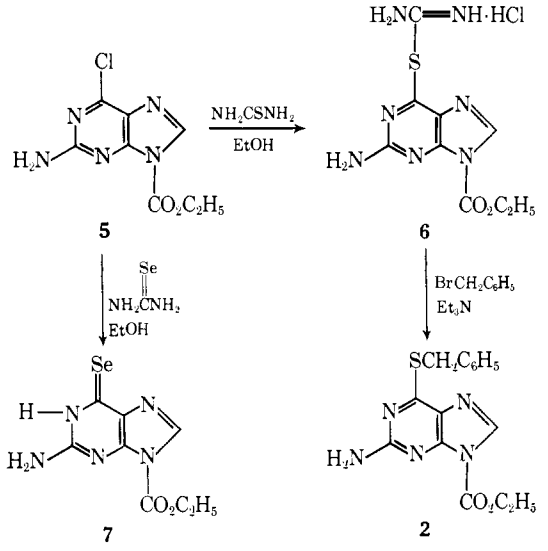


No.	X	R	Method[a]	Yield,[b] %	Mp, °C dec	Formula	Analyses[c]	λ_{max}, mμ	$\epsilon_{max} \times 10^{-3}$
2	$SCH_2C_6H_5$	C_2H_5	A	75	186–188	$C_{15}H_{15}N_5O_2S$	C, H, N	314	12.9
4[d]	$SCH_2C_6H_5$	C_2H_5	A	90	166–168	$C_{17}H_{17}N_5O_3S$	C, H, N	313	23.5
5	Cl	C_2H_5	A	90	200–201	$C_8H_8ClN_5O_2$	C, N; H[e]	312	6.67
6	$SC(=NH)NH_2 \cdot HCl$	C_2H_5	B	77	225–227	$C_9H_{12}ClN_7O_2S$	C, H, N, S	339	12.8
7[f]	SeH	C_2H_5	B	47	184–185	$C_8H_9N_5O_2Se$	C, H, N	373	19.7
8	SCH_3	C_2H_5	A	68	172–174	$C_9H_{11}N_5O_2S$	C, H, N	314	10.4
9	SCH_3	CH_3	A	57	154–155	$C_8H_9N_5O_2S$	C, H, N	312	10.7
10	$SCH_2C_6H_5$	CH_3	A	75	180–181	$C_{14}H_{13}N_5O_2S$	C, H, N	312	10.7
11	Br	C_2H_5	A	79	191–193	$C_8H_8BrN_5O_2$	C, H, N	313	7.37
12	Br	CH_3	A	81	168–170	$C_7H_6BrN_5O_2$	C, H, N	316	7.22
13	Cl	CH_3	A	69	166–167	$C_7H_6ClN_5O_2$	N, H; C[g]	312	7.12

[a] General methods are described in the Experimental Section. [b] Crude yield. All compounds described were recrystallized from EtOH, with the exception of **6** and **7** which were analytically pure as obtained. [c] Analytical results were within ±0.4% of the theoretical values except as indicated. [d] 2-Acetamido. [e] H: calcd, 3.33; found, 3.79. [f] Drawn in the selenol form for convenience. [g] C: calcd, 36.93; found, 37.44.

6-yl)-2-thiopseudourea hydrochloride (**6**) (Scheme II) probably as a result of the $+R$ effect of the 2-amino group, since the reaction of ethyl 6-chloropurine-9-carboxylate has recently been shown to yield ethyl purine-6-thione-9-carboxylate.[18] However, the reaction of selenourea with **5** gave ethyl 2-amino-6-selenopurine-9-carboxylate (**7**) without isolation of the pseudoselenourea hydrochloride. The pseudothiouronium salt (**6**) was easily broken down and alkylated by the action of benzyl bromide and 2 equiv of triethylamine to yield **2**.

SCHEME II

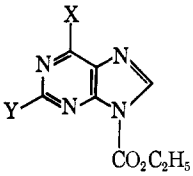


Although the independent synthesis based on the known 2-acetamido-6-benzylthiopurine[6] (**3**) demonstrated that acylation had occurred on the purine ring, not the 2-amino group, assignment to the 9 position was made by analogy to previous work[2,18] on the acylation of purine-6-thione and 2-aminopurine-6-thione which showed the site of reaction to be the 9 position.

Pharmacological tests (Table II) indicate that the

TABLE II

SCREENING TESTS BY CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER



X	Y	Host[a]	Test[b] system	Dose, mg/kg	Survivors	Survival (days)[c] or tulnor wt (g)[d] Test	Control	T/C, %
Cl	NH_2	02	LE	400	4/4	11.0	9.0	122
				200	4/4	9.0	9.0	100
				100	4/4	9.5	9.0	105
				100	6/6	10.3	8.5	121
				50	4/4	9.3	9.0	103
		51	WM	100	6/6	5.3	8.8	60
SCH_3	NH_2	02	LE	400	3/4	11.0	9.0	122
				200	4/4	9.0	9.0	100
				100	4/4	8.8	9.0	97
				100	6/6	10.3	8.5	121
				50	4/4	9.3	9.0	103
		51	WM	100	6/6	8.1	8.8	92
Cl[e]	H	02	LE	500	0/6			
				400	6/6	10.3	9.3	110
				250	6/6	12.2	8.8	138
				250	4/6	13.0	9.0	144
				200	6/6	9.3	8.8	105
				200	6/6	9.5	9.3	102
				100	6/6	10.2	9.3	109
		50	WM	200	2/6	2.5	9.0	Toxic
				100	6/6	3.7	9.3	39
		90	KB	W[f]	Slope = −0.62			
					ED_{50} = 36 μg/ml			

[a] Host: 02, BDF_1; 50, random-bred albino rat; 51, Fischer 344 rat; 90, cell culture. [b] LE, L1210 lymphoid leukemia; WM, Walker carcinosarcoma 256; KB, human epidermoid carcinoma. [c] For LE tests. [d] For WM tests. [e] Preparation of this compound in ref 18. [f] Once a day, 10^5 level.

(18) E. Dyer, R. E. Farris, Jr., C. E. Minnier, and M. Tokizawa, submitted for publication.

9-carbethoxy derivatives of 2-amino-6-chloropurine and of 2-amino-6-methylthiopurine lack significant activity toward L1210 lymphoid leukemia or Walker carcinosarcoma 256. The ethyl 6-chloropurine-9-carboxylate showed some inhibition of L1210 and of Walker carcinosarcoma 256; the substance was inactive toward KB cell culture. None of these compounds was as active an anticancer agent as 2-aminopurine-6-thione,[4] 2-amino-6-chloropurine,[5] or 6-chloropurine.[19]

Experimental Section[20]

Procedure A. Ethyl 2-Amino-6-chloropurine-9-carboxylate (5).—To a stirred solution of 2-amino-6-chloropurine (8.0 g, 48 mmoles) and NaOH (4.0 g, 100 mmoles) in 300 ml of H_2O was added ethyl chloroformate (10.8 g, 100 mmoles). The mixture was stirred for 1 hr, the pH was adjusted to 5 with glacial HOAc, and the precipitate was filtered and dried *in vacuo* to yield 10.3 g (93%) of product; nmr (DMSO-d_6), δ 1.41 (t, 3), 4.52 (q, 2 H), 8.49 (s, 1).

Procedure B. Ethyl 2-Amino-6-selenopurine-9-carboxylate (7).—To a refluxing solution of selenourea (0.102 g, 0.83 mmole) in 20 ml of anhydrous EtOH was added in one portion ethyl 2-amino-6-chloropurine-9-carboxylate (0.2 g, 0.83 mmole). The solution turned yellow and a precipitate appeared in 15–20 min. The solution was refluxed for 45 min more and cooled to room temperature, and the precipitate was filtered, washed with EtOH, and dried *in vacuo* to yield 0.11 g (47%) of analytically pure product.

Ethyl 2-Acetamido-6-benzylthiopurine-9-carboxylate (4) by Acetylation of 2.—A solution of 2 (0.14 g, 0.43 mmole) and Ac_2O (1 ml) in 4 ml of dry toluene was heated under reflux for 1.5 hr. Upon cooling and scratching, a precipitate of colorless crystals deposited which was washed with a small amount of cold Et_2O and dried *in vacuo* to yield 0.09 g (57%) of product: nmr (DMSO-d_6), δ 1.33 (t, 3), 2.4 (s, 3), 4.55 (q, 2), 4.75 (s, 2), 7.42 (m, 5), 8.65 (s, 1). A mixture melting point with **4** obtained by acylation of 2-acetamido-6-benzylthiopurine (**3**) with ethyl chloroformate by procedure A showed no depression and their ir spectra were superimposable.

Independent Synthesis of Ethyl 2-Amino-6-benzylthiopurine-9-carboxylate (2) by Alkylation of 2-(2-Amino-9-carbethoxypurin-6-yl)-2-thiopseudourea Hydrochloride (6).—Benzyl bromide (0.171 g, 1.00 mmole) was added to a stirred solution of **6** (0.317 g, 1.00 mmole) and Et_3N (0.202 g, 2.00 mmoles) in 10 ml of anhydrous DMF. The solution was stirred for 3.5 hr and poured into 50 ml of ice water and the pH was adjusted to 7 with glacial HOAc. The precipitate was filtered and dried *in vacuo* to yield 0.17 g (52%) of product. After recrystallization from EtOH, a mixture melting point with **2** prepared by procedure A was undepressed and their ir spectra were superimposable; nmr (DMSO-d_6), δ 1.42 (t, 3), 4.48 (q, 2), 4.60 (s, 2), 7.40 (m, 5), 8.35 (s, 1).

(19) F. M. Schabel, Jr., J. A. Montgomery, H. E. Skipper, W. R. Laster, Jr., and J. R. Thomson, *Cancer Res.*, **21**, 690 (1961).

(20) Melting points, determined on a Fisher-Johns apparatus, were corrected. Uv spectra were obtained on a Perkin-Elmer 202 spectrophotometer and nmr spectra on a Varian A-60-A instrument.

The Hepatocarcinogenicity of Some Disubstituted 4-Dimethylaminoazobenzenes

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Received July 1, 1968

Many of the disubstituted dimethylaminoazobenzenes (DAB) (Table I) have been tested for rat hepatocarcinogenic activity.[1,2] With the exception of the fluoro derivatives none of the methyl- or halogen-substituted compounds has been more active than DAB itself. In fact preliminary work indicated that disubstituted compounds with the exception of F_2DAB have zero activity on the Miller scale.[1] Later work[2] showed mild carcinogenic activity for 3′,4′-Me_2DAB and we have since verified this activity. We have now shown that 2′,3′-Me_2DAB is extremely active. Since 4′-EtDAB shows greater activity than DAB itself,[2-4] we have extended our work to a related disubstituted compound, 3′,4′-Et_2DAB, and it has been found to be fairly active. Neither 2′,3′-Cl_2 nor 3′,4′-Cl_2DAB was found to have any activity under our testing conditions.

TABLE I: SUBSTITUTED 4-DIMETHYLAMINOAZOBENZENES

Compd	Mp, °C	Yield, crude %	Formula	Analyses
2′,3′-Me_2DAB	120–121	70	$C_{16}H_{19}N_3$	C, H, N
3′,4′-Me_2DAB	Ref 2			
3′,4-Et_2DAB[a]	82–83	25	$C_{18}H_{23}N_3$	C, H, N
2′,3′-Cl_2DAB	218–220	35	$C_{14}H_{13}Cl_2N_3$	C, H, N
3′,4-Cl_2DAB	159–160	55	$C_{14}H_{13}Cl_2N_3$	C, H, N

[a] J. P. Lambooy, *J. Am. Chem. Soc.*, **71**, 3756 (1949).

Experimental Section

All melting points were determined on a Fisher-Johns apparatus and are corrected. The C, H, N analyses were performed in this department on an F and M Model 185 analyzer by Mr. Daryl Sharp. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

N,N-Dimethyl-*p*-(3-*o*-xylylazo)aniline.—2,3-Dimethylaniline hydrochloride (Eastman Kodak) (31 g) was dissolved in a mixture of 80 ml of concentrated HCl and 200 ml of H_2O and diazotized at 0° using 14 g of $NaNO_2$. One-half hour after the final addition a solution of 24 g of $C_6H_5NMe_2$, 200 ml of EtOH, 120 ml of H_2O, and 72 g of NaOAc was added, and the solution was stirred for another 30 min and made basic with NH_4OH. Filtration, washing, and drying afforded the crude azo compound. The others were made in the same way. Crystallization from EtOH and in some cases chromatography on alumina from C_6H_6 gave the pure materials.

Biological Properties.—Young male rats of the Sprague–Dawley strain, approximately 8 weeks old and weighing 150–200 g, were distributed as equally as possible in initial body weight into groups of ten animals each. Each group was fed a diet, patterned after the "low protein, low riboflavin" diet of Miller[1] to which had been added one of the azo compounds at a level of 0.06%. The composition of the basal diet per kilogram was as follows: crude casein, 120 g; cerelose, 770 g; Osborne and Mendel salt mixture, 40 g; corn oil, 50 g; Vitab (rice bran concentrate, obtained from Charles Bowman Co.), 20 g; riboflavin, 0.5 mg; vitamin A palmitate, 67,500 IU.

A group received DAB at the 0.06% while the control group received only the basal diet. All the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment.

Results and Discussion

DAB gave tumor incidences of 6/10 at 4 months and and 9/10 at 6 months. 3′,4′-Me_2DAB gave 0/10 at 2 months, 8/10 at 6 months, and 10/10 gross tumors at 8 months. On the other hand, 2′,3′-Me_2DAB gave 10/10 in 1 month with gross tumors in rats surviving to 2 months, 3′,4′-Et_2DAB gave 0/9 tumors in 4 months,

(1) J. A. Miller and E. C. Miller, *Adcan. Cancer Res.*, **1**, 339 (1953).

(2) J. A. Miller, E. C. Miller, and G. C. Finger, *Cancer Res.*, **17**, 387 (1957).

(3) K. Sugiura, M. L. Crossley, and C. J. Kensler, *J. Natl. Cancer Inst.*, **15**, 67 (1954).

(4) E. V. Brown and A. A. Hamdan, *ibid.*, **27**, 663 (1961).