other absorbing species are produced from each mole of substrate. It was, therefore, concluded that K is of the order of 10^2-10^3 and that the use of eq. 1 to calculate rate constants does not introduce serious difficulties.

Product Spectra.—For all the substrates except isopropylmercuric iodide, the product spectra were essentially those expected if each mole of starting material had produced one-half mole of mercuric iodide. The isopropylmercuric iodide product mixtures seemed to contain some substance absorbing strongly at the shorter wave lengths, although the optical density at 2700 Å. was appropriate. When 2 ml. of product solution was mixed with 1 ml. of 0.4 M sodium iodide, the characteristic spectrum of HgI_4^{--} was obtained, with a peak of the expected intensity at 3220 Å. An identical spectrum was obtained from 2 ml. of mercuric chloride solution of an appropriate concentration mixed with 1 ml. of 0.4 M sodium iodide. These findings support the stoichiometry originally proposed[2] and justify the use of eq. 1 to obtain rate constants.

Acknowledgment.—We are very pleased to acknowledge the financial support of the National Science Foundation, the Air Force Office of Scientific Research, and the Graduate School of the University of Minnesota.

[CONTRIBUTION FROM THE KETTERING-MEYER LABORATORY,[1] SOUTHERN RESEARCH INSTITUTE, BIRMINGHAM 5, ALA.]

Synthesis of Potential Anticancer Agents. XXVI. The Alkylation of 6-Chloropurine[2]

BY JOHN A. MONTGOMERY AND CARROLL TEMPLE, JR.

RECEIVED AUGUST 23, 1960

A new procedure has been developed for the preparation of 9-alkylpurines by the alkylation of 6-chloropurine with a variety of substituted alkyl halides in dimethyl sulfoxide. A lesser amount of the 7-alkylpurines was obtained. These N-substituted-6-chloropurines were converted to a number of derivatives for anticancer screening.

The anticancer activity of 9-alkyl-6-chloro-9H-purines and 9-alkyl-9H-purine-6(1H)-thiones[3] made it desirable to find a shorter route to these compounds. This was particularly true for the synthesis of 8-C^{14}-labeled purines of this type needed for biological studies, since the original synthesis does not lend itself to the conservation of C^{14}. An obvious method of accomplishing this purpose is the direct alkylation of 6-chloropurine, especially since 6-chloropyrine-8-C^{14} has been prepared in this Laboratory.

There are no examples of the alkylation of 6-chloropurine in the literature,[4,6] although purine itself has been methylated in the 9-position by the use of dimethyl sulfate and diazomethane.[14] Other attempted methylation procedures have failed.[15]

Although 6-chloropurine could be methylated with iodomethane in dilute aqueous sodium hydroxide, ethylation of purine or 6-chloropurine with iodoethane under the same conditions was not successful. However, when dimethyl sulfoxide and potassium carbonate were used with iodoethane, reaction took place and 6-chloro-9-ethyl-9H-purine (I) was obtained in 50% yield along with a small amount of 6-chloro-7-ethyl-7H-purine (II). The presence of both isomers was detected by paper chromatography, and the identity of the isomers was established by ultraviolet spectroscopy. Although the spectra of the two 6-chloropurines are very similar, they are easily converted to the corresponding 6-dimethylaminopurines (Ia and IIa) whose spectra are quite different (see Table I). This difference in 6-dimethylamino-7- and -9-alkylpurines was originally observed by Baker, Schaub and Joseph[16] in connection with the synthesis of puromycin.

TABLE I
ULTRAVIOLET SPECTRA

Compound	pH 1 λ_{max}, mμ	pH 1 $\epsilon \times 10^{-3}$	pH 7 λ_{max}, mμ	pH 7 $\epsilon \times 10^{-3}$
6-Dimethylamino-9-ethyl-9H-purine (Ia)[a]	270	17.5	277.5	18.0
6-Dimethylamino-7-ethyl-7H-purine (IIa)[a]	290	19.8	295	17.4
6-Chloro-9-ethyl-9H-purine (I)[b]	265	9.5	266	9.4
6-Chloro-7-ethyl-7H-purine (II)	267	8.0	270	8.1
6-Chloro-7-methyl-7H-purine[c]	268	8.4	271	8.5

[a] See Ref. 16. [b] See Ref. 18. [c] R. N. Prasad and R. K. Robins, *J. Am. Chem. Soc.*, 79, 6401 (1957).

This synthetic method was first applied to the synthesis of 6-chloro-9-ethyl-9H-purine-8-C^{14} from which 9-ethyl-9H-purine-6(1H)-thione-8-C^{14} was

(1) Affiliated with the Sloan-Kettering Institute.

(2) This work was presented at the Southeastern Regional Meeting of the American Chemical Society, Richmond, Va., November 4, 1959. It was supported by funds from the C. F. Kettering Foundation and from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740.

(3) H. E. Skipper, J. A. Montgomery, J. R. Thomson and F. M. Schabel, Jr., *Cancer Research*, **19**, 425 (1959).

(4) In a preliminary account of her work on N-methylpurines, Elion mentioned the methylation and conversion of 6-chloropurine to 7-methyl-7H-purine-6(1H)-thione and 9-methyl-9H-purine-6(1H)-thione. No description of the N-alkyl-6-chloropurines is given.[5]

(5) G. B. Elion in G. W. E. Wolstenholme and C. M. O'Connor, eds., "The Chemistry and Biology of Purines," (A Ciba Foundation Symposium), J. and A. Churchill, Ltd., London, 1957, p. 39.

(6) A number of 6-chloropurine nucleosides[7-12] have been prepared by application of the mercuri procedure[13] to this purine. The procedure failed with the less reactive iodoethane.

(7) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1959).

(8) H. M. Kissman and M. J. Weiss, *J. Org. Chem.*, **21**, 1053 (1956).

(9) L. Goodman, J. W. Marisco and R. B. Angier, *J. Am. Chem. Soc.*, **78**, 4173 (1956).

(10) B. R. Baker, K. Hewson, H. J. Thomas and J. A. Johnson, *J. Org. Chem.*, **22**, 954 (1957).

(11) R. E. Schaub, M. J. Weiss and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 4692 (1958).

(12) F. J. McEvoy, B. R. Baker and M. J. Weiss, *ibid.*, **82**, 209 (1960).

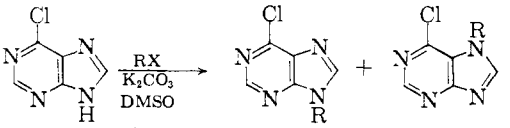
(13) J. Davoll and B. A. Lowy, *ibid.*, **73**, 1650 (1951); J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *ibid.*, **78**, 2117 (1956).

(14) H. Bredereck, H. Ulmar and H. Waldmann, *Chem. Ber.*, **89**, 12 (1956).

(15) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

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

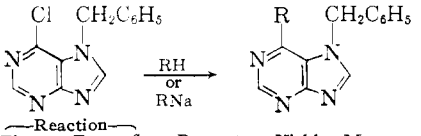
TABLE II



Compound	RX	Reaction Time, hr.	Reaction Temp., °C.	Molar ratio of reactants[a]	Yield of 9-isomer, %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
I	C_2H_5I	2	26	2.0	50	81–84	46.00	45.87	3.84	3.80	30.70	30.46
II					4.7[b]	122–123	46.00	45.90	3.84	3.91	30.70	30.52
III	$HOCH_2CH_2Br$	1.5	90	2.0[c]	20[d]	154–157	42.32	42.29	3.53	3.57	28.21	28.12
IV	$CH_3COOCH_2CH_2Br$	15	26	1.4[e]	46	74–76	44.91	45.19	3.74	3.79	23.29	23.44
V	$C_6H_5CH_2Cl$	3	26	2.0	38	86–87	58.90	58.76	3.68	3.92	22.91	22.84[f]
VI					15[b]	152–153	58.90	58.77	3.68	3.90	22.91	22.94
VII	$ClCH_2CH_2Br$	2.5	26	1.1	19	108	38.70	38.75	2.76	2.81	25.80	25.94[g]
VIII	$NCCH_2Cl$	1	50	1.2	35	134–135	43.40	43.36	2.07	2.08	36.17	35.86
IX	$C_2H_5OOCCH_2Br$	2	26	1.1	35	93–95	44.91	44.85	3.74	3.89	23.29	23.18

[a] RX/6-chloropurine. [b] 7-Isomer. [c] 1:1 ratio at start, 0.5 equivalent of halide added at end of 0.5 hr. and another 0.5 at the end of 1 hr. [d] Contaminated with some 7-isomer. [e] 1:1 ratio at start, 0.4 equivalent added at end of 10 hr. [f] Calcd.: Cl, 14.52. Found: Cl, 14.64. [g] Calcd.: Cl, 32.50. Found: Cl, 32.64.

TABLE III



Compound	R	Reagent	Reaction Time, hr.	Reaction Temp.,[a] °C.	Recrystn. solvent	Yield, %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
VIa	OH	1 *N* NaOH	1	A	H_2O[b]	93	>260	63.70	63.65	4.46	4.63	24.77	24.61
b	OCH_3	CH_3ONa, CH_3OH	2	A	H_2O[b]	88	126–127	64.98	64.48	5.03	4.98	23.32	23.15
c	NHC_2H_5	70% aq. $C_2H_5NH_2$	1	A	1 *N* HCl	74	249–250[c]	58.00[d]	57.51	5.53[d]	5.53	24.20[d]	24.23
d	$NHC_6H_4Cl(p)$	*p*-$ClC_6H_4NH_2$, PrOH	2	A	Ether[b]	72	234–235	58.10	57.95	4.03	4.16	18.80	18.72
e	$NHNH_2$	95% N_2H_4	0.25	B	H_2O[b]	88	.....[e]	59.98	59.90	5.03	5.12	34.98	34.96
f	NH_2	Alc. NH_3[f]	16	125–130[g]	H_2O	87	238–239[c]	59.25[h]	59.16	5.39[h]	5.39	28.79[h]	28.43
g	H	H_2, Pd/C	0.3	B	H_2O	68	145–146	68.55	68.51	4.79	4.75	26.65	26.93
h	SH	$(H_2N)_2CS$, PrOH	2	B	1 *N* NaOH[i]	57	265–266	59.50	58.99	4.16	4.06	23.13	23.34
i	$N(CH_3)_2$[j]	25% aq. $(CH_3)_2NH$	1.5	A	Bz–Sk. C[k]	77	134–135	66.38	65.72	5.97	5.84	27.65	27.43

[a] A, boiling point of solution; B, room temperature. [b] Trituration. [c] With decomposition. [d] Calcd. for $C_{14}H_{15}N_5 \cdot HCl$. [e] Indefinite. [f] Saturated at 0°. [g] Bomb. [h] Calcd. for $C_{12}H_{11}N_5 \cdot H_2O$. [i] Precipitated by neutralization with acetic acid. [j] See ref. 16. [k] Benzene–Skellysolve C (1:2).

prepared by reaction with thiourea. The scope of the reaction was then broadened by applying it to α-chlorotoluene and a number of other substituted alkyl halides (see Table II). The yield of pure 9-isomer obtained varied between 19 and 50%. This variation can be attributed in a large part to the difficulties encountered in the purification of the different compounds. In one case, that of 6-chloro-9-(2-hydroxyethyl)-9*H*-purine (III), we were unable to free the 9-isomer completely of the 7-isomer. However, the acetyl derivative of III, 2-(6-chloro-9*H*-purin-9-yl)-ethyl acetate (IV), was isolated free from the 7-isomer with little difficulty. The 7- and 9-benzyl-6-chloropurines (V and VI) were readily separated and, in this case, the yield of pure 7-isomer obtained was large enough to permit the preparation of a number of 7-benzyl-7*H*-purines by the nucleophilic displacement of its 6-chlorine atom in the usual manner. The 7- and 9-isomers were identified by conversion to the known 7-benzyl-6-dimethylamino-7*H*-purine[16] and 9-benzyl-6-dimethylamino-9*H*-purine as described above for the N-ethyl compounds. In the other cases investigated, the 9-isomers (VII-IX) were obtained pure and, although a small amount of the 7-isomer was probably present in all cases, none was isolated.

The N-substituted 6-chloropurines were treated with a number of nucleophilic reagents in the usual manner[17,18] to prepare derivatives for testing as potential anticancer agents. Table III lists the 7-benzyl-6-substituted-7*H*-purines prepared, and Table IV the 6,9-disubstituted-9*H*-purines.

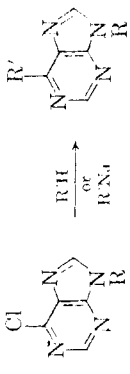
Anomalous ultraviolet spectral data obtained with 9-benzyl-6-chloro-9*H*-purine caused us to investigate the behavior of 6-chloro-9-ethyl-9*H*-purine in 0.1 *N* sodium hydroxide containing small amounts of methanol. Whereas the chloropurine remained unchanged in 0.1 *N* sodium hydroxide itself (at room temperature), the addition of as little as 0.2% methanol caused a significant amount of 9-ethyl-6-methoxy-9*H*-purine to be formed in 4 hours, and 10% methanol caused complete conversion to the methoxypurine to take place in 1 hour (at room temperature). Even though it is known that the addition of water, up to 50% by volume, to solutions of methoxide in methanol increases the rate of methoxylation and that the equilibrium of eq. I lies far to the right,[19] it is somewhat surprising that such small amounts of meth-

(17) A. Bendich, P. J. Russell and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1957).

(18) J. A. Montgomery and C. Temple, Jr., *ibid.*, **79**, 5238 (1957).

(19) J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951).

TABLE IV



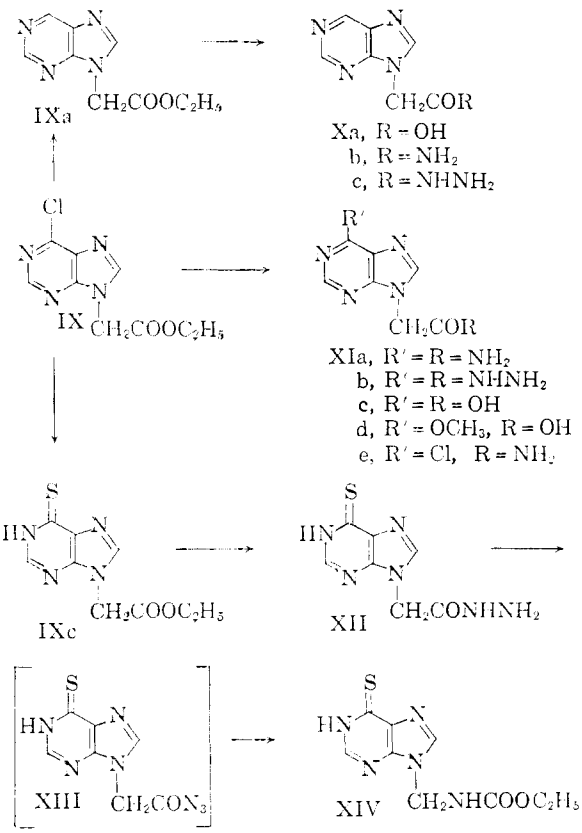
Compound	R'	R	Reagent	Reaction Time, hr.	Reaction Temp.,[a] °C.	Recrystn. solvent	Yield, %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Va	OH	$CH_2C_6H_5$	1 *N* HCl	1	A	H_2O[b]	87	>260	63.70	63.57	4.46	4.54	24.77	24.56
a	OH	$CH_2C_6H_5$	2 *N* NaOH	1	A	H_2O[b]	69[c]							
b	OCH_3	$CH_2C_6H_5$	CH_3ONa, CH_3OH	1	A	H_2O[b]	96	128	64.98	64.98	5.03	5.13	23.32	23.36
c	NH_2	$CH_2C_6H_5$	Alc. NH_3[d]	16	130°	Alc.–H_2O	73	235	63.98	63.60	4.92	4.84	31.09	31.16
d	NHC_2H_5	$CH_2C_6H_5$	70% aq. $C_2H_5NH_2$	2	A	1 *N* HCl	73	...[e]	58.00[f]	57.52	5.53[f]	5.55	24.20[f]	24.10
e	$NHC_6H_4Cl(p)$	$CH_2C_6H_5$	*p*-$ClC_6H_4NH_2$, PrOH	1	A	Ether[b]	82	195–196	58.10[g]	57.92	4.03[g]	4.19	18.80[g]	18.79
f	$NHNH_2$	$CH_2C_6H_5$	95% N_2H_4	0.2	B	H_2O[b]	91	209–210	59.98	60.32	5.03	5.15	34.98	34.68
g	H	$CH_2C_6H_5$	H_2, Pd/C	0.25	B	Ether	85	100–101	68.55	68.46	4.79	5.14	26.65	26.43
h	SH	$CH_2C_6H_5$	$(H_2N)_2CS$, PrOH	2	A	PrOH[b]	93	>260	59.50	59.77	4.16	4.50	23.13	23.26
i	$N(CH_3)_2$	$CH_2C_6H_5$	25% aq. $(CH_3)_2NH$	1	A	Sk. C[h]	77	131–132	66.38	66.04	5.97	6.10	27.65	27.35
IXa	H	CH_2COOEt	H_2, Pd/C	...	B	Sk. C[h]	81	121–122	52.42	52.76	4.89	5.18	27.17	27.22
b	$NHC_6H_4Cl(p)$	CH_2COOEt	*p*-$ClC_6H_4NH_2$, PrOH	1	A	Sk. C[h]	64	184–185	54.31	54.04	4.22	4.34	21.10	21.50
c	SH	CH_2COOEt	$(H_2N)_2CS$, EtOH	1	A	EtOH	88	>260	45.38	45.17	4.23	4.46	23.52	23.41
XIIf	SH	CH_2CONH_2	$(H_2N)_2CS$, EtOH	1	A	0.3 *N* NaOH[i]	81	>260	39.40[j]	39.39	3.50[j]	3.61	32.85[j]	32.78

[a] A, boiling point of the solution. B, room temperature. [b] Trituration. [c] In this reaction a 16% yield of 5-amino-4-benzylamino-6-chloropyrimidine was also obtained, m.p. 201–202°. *Anal.* Calcd. for $C_{11}H_{11}ClN_4$: C, 56.20; H, 4.68; N, 23.85. Found: C, 55.83; H, 4.74; N, 23.36. [d] Saturated at 0°. [e] Indefinite. [f] Calcd. for $C_{14}H_{15}N_5 \cdot HCl$. [g] Calcd. for $C_{18}H_{14}ClN_5 \cdot HCl$. [h] Skellysolve C. [i] Precipitated by neutralization with acetic acid. [j] Calcd. for $C_7H_7N_5OS \cdot {}^1/_4H_2O$.

anol cause such a ready displacement of the chlorine atom of these 6-chloropurines.

$$CH_3OH + OH^- \longrightarrow CH_3O^- + H_2O \qquad (I)$$

With ethyl (6-chloro-9*H*-purin-9-yl)acetate (IX), displacement of the C_6-chlorine atom and reaction of the ester group took place simultaneously in some cases. Thus, reaction with ammonia



in the usual manner gave 6-amino-9*H*-purine-9-acetamide (XIa) and with hydrazine, 6-hydrazino-9*H*-purine-9-acetic acid hydrazide (XIb). 6-Chloro-9*H*-purine-9-acetamide (XIe) could be prepared from IX by reaction in liquid ammonia. Treatment of IX with 1 *N* hydrochloric acid gave 6-hydroxy-9*H*-purine-9-acetic acid (XIc), while treatment with sodium methoxide in methanol gave 6-methoxy-9*H*-purine-9-acetic acid (XId). Reduction of IX in the usual manner[20] gave ethyl 9*H*-purine-9-acetate (IXa), which was converted to the acid Xa, the amide Xb and the acid hydrazide Xc. Isolation and purification of 9*H*-purine-9-acetic acid proved difficult owing to its water solubility. Hydrolysis with barium hydroxide followed by removal of the barium ions

(20) Triethylamine was found to work well as the acid acceptor in place of the customary magnesium oxide.[17,18]

with sulfuric acid finally provided the pure material.

Ethyl (6-mercapto-9*H*-purine-9-yl)-acetate was methylated in the usual manner to give ethyl [6-(methylthio)-9*H*-purin-9-yl]-acetate (XV) and hydrolyzed in 1 *N* sodium hydroxide solution to give 6-mercapto-9*H*-purine-9-acetic acid (XVI), which, being much less water soluble than 9*H*-purine-9-acetic acid, was isolated pure with no difficulty.

6-Mercapto-9*H*-purine-9-acetic acid hydrazide (XII), prepared from ethyl (6-mercapto-9*H*-purin-9-yl)-acetate (IXc) and hydrazine on treatment with aqueous sodium nitrite, gave the acid azide XIII. Although this compound was not obtained analytically pure, its identity was firmly established by its infrared spectrum and by its conversion to the corresponding carbamate, ethyl N-(6-mercapto-9*H*-purin-9-yl)-carbamate (XIV). The spectrum of the acid azide XIII showed the disappearance of primary NH stretching bands (3210 cm.$^{-1}$) and secondary NH stretching bands (3160 and 3060 cm.$^{-1}$) present in the spectrum of XII, the expected shift in the carbonyl frequency of XII from 1665 to 1720 cm.$^{-1}$, and finally the appearance of the N≡≡N band at 2150 cm^{-1}. The identity of the carbamate was established by its spectra and elemental analyses.

None of the compounds tested thus far in the primary screen of the Cancer Chemotherapy National Service Center have shown any significant anticancer activity.

Acknowledgment.—The authors are indebted to Mr. W. E. Fitzgibbon and the Organic Preparations Section of Southern Research Institute who carried out the large scale syntheses of some of the compounds and to Dr. W. J. Barrett and the Analytical Section of Southern Research Institute who performed the spectral and most of the microanalytical determinations reported. Some of the analyses reported were performed by the Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Experimental

The ultraviolet absorption spectra were determined in aqueous solution with a Beckman DK-2 spectrophotometer, but the optical densities at the maxima were determined with a Beckman DU. The infrared spectra were determined in pressed potassium bromide disks with a Perkin–Elmer model 21 spectrophotometer. Melting points were determined on a Kofler Heizbank and are corrected.

The Alkylation of 6-Chloropurine. The Reaction.—6-Chloropurine and the alkyl halide were dissolved in dimethyl sulfoxide (10 ml./mmole of 6-chloropurine) and anhydrous potassium carbonate (1.0 to 1.1 mmoles per mmole of 6-chloropurine) added. After reaction was complete as indicated by the ultraviolet spectrum of time aliquots (see Table II for reaction time and temperature), the product was isolated as indicated below.

Isolation Procedure A.—The reaction mixture was cooled, diluted with water (6 ml./mmole of 6-chloropurine) and processed as follows:

6-Chloro-9-(2-hydroxyethyl)-9H-purine (III).—The solution was extracted with ether in liquid–liquid extractor for 36 hours. Evaporation of the ether extract gave a gum which was recrystallized from benzene to give the analytical sample (see Table II).

Ethyl (6-Chloro-9H-purin-9-yl)-acetate (IX).—The solution was extracted six times with portions of ether (8 ml./mmole of 6-chloropurine), which were combined, dried, and evaporated to dryness. The residue was recrystallized from cyclohexane (24 ml./mmole of 6-chloropurine) and then from Skellysolve B (48 ml./mmole of 6-chloropurine) to obtain the analytical sample (see Table II).

6-Chloro-9-ethyl-9H-purine and 6-Chloro-7-ethyl-7H-purine (I and II).—The solution was extracted 3 times with portions of ether (5 ml./mmole of 6-chloropurine) which were combined, dried, and evaporated to dryness. The residue was recrystallized from Skellysolve C (5 ml./mmole) giving pure 6-chloro-9-ethyl-9*H*-purine. The aqueous solution described above was then extracted three times with portions of benzene (4 ml./mmole), which were combined, dried, and evaporated to dryness. The residue was extracted with ether (to remove 9-isomer) and then recrystallized from Skellysolve C giving pure 6-chloro-7-ethyl-7*H*-purine (see Table II).

9-Benzyl-6-chloro-9H-purine and 7-benzyl-6-chloro-7H-purine (V and VI).—The oil that separated from the water solution obtained as described above was removed and triturated with ether. The solid that deposited was extracted with Skellysolve C (1 ml./mmole) and then recrystallized from water giving pure 7-benzyl-6-chloro-7H-purine. The ether and Skellysolve C extracts were combined, dried, and evaporated to dryness. The residue was recrystallized twice from Skellysolve C to give pure 9-benzyl-6-chloro-9H-purine (see Table II).

Isolation Procedure B.—The insoluble material in the reaction mixture was removed by filtration, washed with dimethyl sulfoxide, and discarded. The combined filtrate and washings were distilled *in vacuo* to remove the dimethyl sulfoxide. The residue was treated as described below.

6-Chloro-9-(2-chloroethyl)-9H-purine (VII).—The residue was extracted with four portions of ether (4 ml./mmole) which were combined and evaporated to dryness. Recrystallization of this residue once from cyclohexane and twice from Skellysolve C gave an analytical sample (see Table II).

6-Chloro-9H-purine-9-acetonitrile (VIII).—The gum obtained as described above was dissolved in water (5 ml./mmole), and the solution was extracted three times with portions of chloroform (1.7 ml./mmole) and discarded. The combined extracts were evaporated to dryness and the resulting residue triturated with ether and then recrystallized from a 1:1 mixture of benzene and cyclohexane to give the analytical sample (see Table II).

2-(6-Chloro-9H-purin-9-yl)-ethyl Acetate (IV).—The residue obtained as described above was extracted four times with portions of ether (2 ml./mmole) which were combined and evaporated. The residual oil was dissolved in water (0.5 ml./mmole), and the resulting solution extracted six times with portions of ether (2 ml./mmole), which were combined, dried, and evaporated. Recrystallization of the residue from Skellysolve C (24 ml./mmole) gave the analytical sample (see Table II).

9H-Purine-9-acetic Acid (Xa).—A solution of ethyl (9H-purin-9-yl 1)-acetate (2.00 g., 9.71 mmoles) in a saturated solution of barium hydroxide (50 ml.) was allowed to stand at room temperature for 4 hours. After the solution was neutralized with 1 *N* sulfuric acid, 0.191 *N* sulfuric acid (51 ml., 9.75 mmoles) was added, the mixture heated to boiling, treated with charcoal, and the insoluble residue removed by filtration through Celite. The filtrate was evaporated to dryness *in vacuo*, the residue triturated with ethanol (20 ml.), and the insoluble material collected by filtration and dried *in vacuo* over P_2O_5: yield 1.36 g. (78.5%), m.p. 251–254° dec.

A small sample of the above solid was recrystallized from ethanol; m.p. 256–257° dec., spectral data; λ_{max} in mμ ($\epsilon \times 10^{-3}$): (1) *p*H 1, 262 (5.85); *p*H 7, 264(7.45); *p*H 13, 264-(7.65); $\bar{\nu}$ in cm^{-}: 2940 (aliphatic CH), 2700–2400 (acidic H), 1710 (C=O), 1600, 1580 and 1510 (C=C, C=N).

Anal. Calcd. for $C_7H_6N_4O_2$: C, 47.19; H, 3.39; N, 31.45. Found: C, 47.24; H, 3.78; N, 31.03.

9H-Purine-9-acetamide (Xb).—A solution of ethyl (9H-purin-9-yl)-acetate (1.12 g., 5.43 mmoles) in ethanolic ammonia (50 ml., saturated at 1°) was heated in a stainless steel bomb at 110–115° for 16 hours. The bomb was opened, the mixture concentrated to 25 ml. in a steam of nitrogen, and the solid collected by filtration, washed with ethanol (10 ml.), and dried *in vacuo* over P_2O_5: yield 680 mg. (71 %), m.p. 245° dec.; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 263 (5.95); *p*H 7, 263 (7.6); *p*H 13, 263 (7.75); $\bar{\nu}$ in cm.$^{-1}$: 3370 and 3180 (amide NH), 2930 (aliphatic CH), 1680 (amide CO), 1590 (amide NH), 1510 (C=C, C=N).

Anal. Calcd. for $C_7H_7N_5O$: C, 47.45; H, 3.98; N, 39.53. Found: C, 47.59; H, 4.40; N, 39.16.

An additional 170 mg. of impure product was obtained from the combined filtrate and wash; m.p. 222° dec. with presoftening.

9H-Purine-9-acetic Acid Hydrazide (Xc).—A solution of ethyl-(9H-purin-9-yl)-acetate (500 mg., 2.43 mmoles) in 95% hydrazine (5 ml.) was stirred for 1 hour at room temperature, diluted with ethanol (20 ml.) and the resulting mixture allowed to stand for 1 hour. The white solid that deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 270 mg. (58%), m.p. 177–178° dec. The analytical sample was obtained by recrystallization of a small sample of this material from ethanol; m.p. 177°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 263 (5.8); *p*H 7, 263 (7.7); *p*H 13, 264 (7.8); $\bar{\nu}$ in cm.$^{-1}$: 3320 and 3160 (NH), 3060 (CH), 1670 (CO), 1590 and 1640 (NH), 1610, 1545 and 1515 (C=C, C=N).

Anal. Calcd. for $C_7H_8N_6O$: C, 43.75; H, 4.20; N, 43.73. Found: C, 43.97; H, 4.42; N. 43.79.

Evaporation of the ethanol–hydrazine filtrate gave an additional 130 mg. of impure product, m.p. 160–170°.

6-Amino-9H-purine-9-acetamide (XIa).—A solution of ethyl (6-chloro-9*H*-purin-9-yl)-acetate (2.00 g., 8.30 mmoles) in ethanolic ammonia (50 ml., saturated at 1°) was heated in a stainless steel bomb at 120–125° for 18 hours. The bomb was then chilled and opened and the tan solid collected by filtration. Recrystallization of this solid from water (200 ml.) gave 1.13 g. (65%) of the hydrate: m.p. >264°. The hydrate did not lose its water of crystallization *in vacuo* at room temperature over P_2O_5; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 258 (14.4); *p*H 7, 260 (14.6); *p*H 13, 260 (14.6); $\bar{\nu}$ in cm.$^{-1}$: 3330 and 3130 (NH), 1675 (amide C=O), 1650 and 1615 (NH), 1580 and 1525 (C=C, C=N).

Anal. Calcd. for $C_7H_8N_6O \cdot H_2O$: C, 40.00; H, 4.76; N, 40.00. Found: C, 39.77; H, 4.93; N, 39.89.

6-Hydrazino-9H-purine-9-acetic Acid Hydrazide (XIb).—Solid ethyl (6-chloro-9H-purin-9-yl)-acetate (1.00 g., 4.16 mmoles) was added portionwise with stirring over a 5-minute period to 95% hydrazine (5.0 ml.). Before the addition was complete a large quantity of solid had deposited from the warm (55°) mixture. The mixture was stirred for an additional 20 minutes, the solid collected by filtration and washed with chloroform (2 × 10 ml.). Recrystallization of this sample from water (40 ml.) with Norit treatment gave 470 ml. (51%) of product, m.p. > 264°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 261 (16.7); *p*H 7, 265 (14.9); *p*H 13, unstable; $\bar{\nu}$ in cm.$^{-1}$: 3325 and 3220 (broad) (NH), 2920 (aliphatic CH), 1690 (amide C=O), 1610 and 1580 (NH), 1570 (shoulder), 1550 (shoulder) and 1520 (C=C, C=N), 1480 and 1400 (unidentified).

Anal. Calcd. for $C_7H_{10}N_8O$: C, 37.84; H, 4.50; N, 50.45. Found: C, 37.45; H, 4.45; N, 50.32.

An additional 40 mg. of product was obtained from the hydrazine filtrate. The total yield was 510 mg. (55%).

6-Hydroxy-9H-purine-9-acetic Acid (XIc).—A solution of ethyl (6-chloro-9*H*-purin-9-yl)-acetate (1.00 g., 4.16 mmoles) in 1 *N* hydrochloric acid (10 ml.) was refluxed for 1 hour, the solution cooled, and 5 *N* sodium hydroxide added carefully (*p*H > 1) until a solid deposited. The solid was collected by filtration, washed with water (5 ml.) and dried *in vacuo* over P_2O_5; yield 540 mg., m.p. 264°. A carbon and hydrogen analysis indicated that this sample was at least 98% pure.

Recrystallization of the above sample (200 mg.) from water (15 ml.) gave the pure material; yield 150 mg., m.p. >264°; spectral data, λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 249 (11.6); *p*H 7, 250 (12.0); *p*H 13, 254 (12.6); $\bar{\nu}$ in cm.$^{-1}$: 3200 (CH), 2900–2500 (acidic H), 1730 (C=O of acid), 1675 (C=O of purine), 1595, 1560 and 1530 (C=C, C=N); 920 (CH of ring).

Anal. Calcd. for $C_7H_8N_4O_3$: C, 43.30; H, 3.12; N, 28.86; Found: C, 43.26; H, 3.43; N, 28.77.

An additional 120 mg. of product was obtained from the work-up of the acidic filtrate.

6-Methoxy-9-H-purine-9-acetic Acid (XId).—A solution of ethyl (6-chloro-9H-purin-9-yl)-acetate (1.00 g., 4.16 mmoles) in methanol (20 ml.) containing sodium methoxide (500 mg., 9.26 mmoles) was refluxed for 1 hour, acidified (*p*H > 1) with 3 *N* hydrochloric acid, and the whole evaporated to dryness. The residue was triturated with 0.1 *N* hydrochloric acid (25 ml.) and dried *in vacuo* over P_2O_5; yield 420 mg. (48.5%), m.p. 246–248° dec. when taken fast from 200°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 251 (10.6); *p*H 7, 251 (11.1); *p*H 13, 251 (11.1); $\bar{\nu}$ in cm.$^{-1}$: 2980 and 2930 (aliphatic CH), 2700–2400 (acidic H), 1720 (C=O), 1600, 1540 and 1490 (C=C, C=N).

Anal. Calcd. for $C_8H_8N_4O_3$: C, 46.15; H, 3.87; N, 26.92. Found: C, 45.79; H, 3.89; N, 26.83.

6-Chloro-9H-purine-9-acetamide (XIe).—A solution of ethyl (6-chloro-9*H*-purin-9-yl)-acetate (1.00 g., 4.16 mmoles) in liquid ammonia (50 ml.) was allowed to reflux under a Dry Ice–acetone condenser for 4 hours. The ammonia was allowed to evaporate, the residue boiled in dioxane (75 ml.), and a small amount of insoluble material removed by filtration. The filtrate was refrigerated, the solid that deposited collected by filtration, washed with ether (5 ml.) and dried *in vacuo* over P_2O_5 at 80°; yield 490 mg, m.p. 229–231° dec. The ultraviolet spectrum of this material indicates that it is unstable in 0.1 *N* sodium hydroxide; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 265 (9.1); *p*H 7, 265 (9.2); $\bar{\nu}$ in cm.$^{-1}$: 3370 and 3140 (NH of amide), 1665 (C=O of amide), 1600, 1570 and 1510 (C=C, C=N).

Anal. Calcd. for $C_7H_6ClN_5O$: C, 39.70; H, 2.84; N, 33,10; Cl, 16.80. Found: C, 39.82; H, 3.14; N, 33.03; Cl, 16.55.

Concentration of the dioxane filtrate gave an additional 160 mg. of impure product, m.p. 216–218° dec.

6-Mercapto-9H-purine-9-acetic Acid Hydrazide (XII).—Solid ethyl (6-mercapto-9*H*-purin-9-yl)-acetate (1.00 g., 4.20 mmoles) was carefully added with stirring to 95% hydrazine (5.0 ml.). The resulting warm solution was stirred at room temperature for 15 minutes, then diluted with water (25 ml.) and neutralized to *p*H 5 with acetic acid. The solid that deposited was collected by filtration, washed with water (25 ml.) and dried *in vacuo* over P_2O_5; yield 750 mg. (80 %), m.p. > 264°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 321 (23.6); *p*H 7, 318 (22.0); *p*H 13, 310 (22.1); $\bar{\nu}$ in cm.$^{-1}$: 3290 (NH), 2920 (aliphatic CH), 1650 (CO), 1605 (NH), 1595, 1560 and 1490 (C=C, C=N).

Anal. Calcd. for $C_7H_8N_6OS$: C, 37.50; H, 3.60; N, 37.49. Found: C, 37.38; H, 3.84; N, 37.56.

Ethyl N-(6-Mercapto-9H-purin-9-ylmethyl)-carbamate (XIV).—To a suspension of 6-mercapto-9*H*-purine-9-acetic acid hydrazide (410 mg., 1.83 mmoles) in 0.1 *N* hydrochloric acid (18.0 ml., 1.84 mmoles) at 24° was added with stirring a solution of sodium nitrite (130 mg., 1.88 mmoles, in 2 ml. of water). After the solution was stirred for 15 minutes the insoluble material was collected by filtration, washed with water and dried; yield 350 mg. When heated, this material exploded at 142–144°.

The azide described above (290 mg.) was suspended in ethyl alcohol (20 ml.), the suspension refluxed for 4 hours, filtered, and evaporated to dryness *in vacuo*. The solid residue (130 mg.) was recrystallized from alcohol; yield 31 mg. (10%), m.p. 217°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 322 (22.0); *p*H 7, 319 (22.2); *p*H 13, 310 (22.2); $\bar{\nu}$ in cm.$^{-1}$: 3300 (NH), 2800 (CH), 2650 (acidic H), 1720 (C=O of NHCOO), 1595, 1540, 1520 (C=C, C=N), 1190 (C—O—), 960 (CH).

Anal. Calcd. for $C_9H_{11}N_5O_2S$: C, 42.69; H, 4.38; N, 27.67. Found: C, 42.17; H, 4.47; N, 27.54.

Ethyl [6-(Methylthio)-9H-purin-9-yl]-acetate (XV).—To a suspension of ethyl (6-mercapto-9*H*-purin-9-yl)-acetate (2.00 g., 8.40 mmoles) in dimethyl sulfoxide (20 ml.) containing anhydrous potassium carbonate (1.19 g., 8.40 mmoles) was added iodomethane (2.0 ml.) and the whole stirred at room temperature for 1 hour.

The mixture was diluted with water (80 ml.) and the solid that deposited was collected by filtration, washed with water (30 ml.) and dried *in vacuo* over P_2O_5; yield 1.14 g. (54%), m.p. 118–119°. The analytically pure sample was obtained by recrystallization of a small portion of the above solid from cyclohexane; m.p. 119°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 293 (17.4); *p*H 7, 292 (broad) (18.9); *p*H 13, 287 (broad) (18.5); $\bar{\nu}$ in cm.$^{-1}$: 2985 (aliphatic CH), 1755 and 1745 (ester C=O), 1575 and 1500 (C=C, C=N), 1450 and 1390 (C—CH_3), 1230 and 1200 (ester C—O—).

Anal. Calcd. for $C_{10}H_{12}N_4O_2S$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.96; H, 4.97; N, 22.36.

6-Mercapto-9H-purine-9-acetic Acid (XVI).—Ethyl (6-mercapto-9*H*-purin-9-yl)-acetate (1.00 g., 4.20 mmoles)

was dissolved in 1 *N* sodium hydroxide (10 ml.), and after standing at room temperature for 30 minutes, the solution was neutralized with dilute sulfuric acid. The solid that deposited was collected by filtration, washed with water (2 × 5 ml.), then acetone (25 ml.), and dried *in vacuo* over P_2O_5; yield 830 mg. (94%), m.p. > 264°; spectral data: λ_{max} in mμ ($\epsilon \times 10^{-3}$): *p*H 1, 225 (9.6), 323 (22.6); *p*H 7, 228 (10.5), 320 (22.2); *p*H 13, 233 (14.3), 310 (22.0); $\tilde{\nu}$ in cm.$^{-1}$: 3100 and 3030 (CH), 2900–2500 (acidic H), 1700 (C=O of COOH), 1610 and 1560 (C=C, C=N), 1205 (C—O of COOH).

Anal. Calcd. for $C_7H_6N_4O_2S$: C, 40.00; H, 2.88; N, 26.66. Found: C, 39.87; H, 3.04; N, 26.94.

[CONTRIBUTION FROM THE RESEARCH AND DEVELOPMENT DIVISION, SMITH KLINE AND FRENCH LABORATORIES, PHILADELPHIA 1, PENNA.]

The Chemistry of Hortiamine and 6-Methoxyrhetsinine[1]

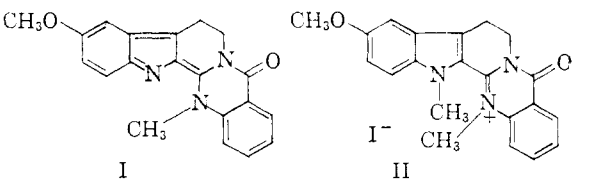
BY IRWIN J. PACHTER, RICHARD J. MOHRBACHER AND DAVID E. ZACHARIAS

RECEIVED AUGUST 22, 1960

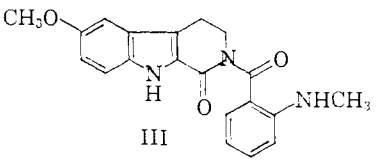
The structure recently assigned to 6-methoxyrhetsinine (III) on the basis of spectral considerations receives support from the nature of its chemical reaction products. The methylation, ethanolysis, lithium aluminum hydride reduction and acetylation of III were studied. The acetylation reaction gives rise to a compound XXVI which is isomeric with hortiamine (I) and which has structural features in common with a hitherto unreported type of rutaceous alkaloid isolated from *Hortia braziliana* Vel. The lithium aluminum hydride reduction of hortiamine (I) was investigated. A new synthesis of hortiamine is described.

In a previous paper,[2] the hypotensive red alkaloid hortiamine was shown to have structure I. Upon methylation it yielded a yellow methiodide (II) and upon hydrolysis it yielded a yellow compound which, on the basis of its spectral properties, was assigned structure III.[3] Compound III is the 6-methoxy derivative of the alkaloid rhetsinine, recently isolated from *Xanthoxylum rhetsa*[4–6] and from *Evodia rutecarpa*.[7]

The present paper describes some new reactions of hortiamine (I) and 6-methoxyrhetsinine (III) and serves to confirm the structures previously advanced for rhetsinine[5] and its 6-methoxy derivative.[2]

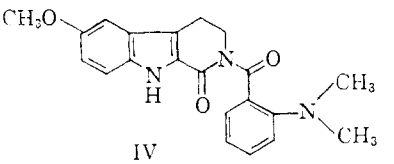


I II

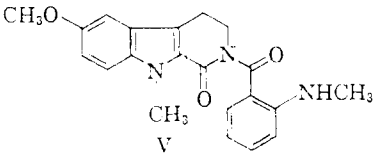


III

When 6-methoxyrhetsinine (III) is methylated with methyl iodide in dimethylformamide, the yellow dimethylamino compound IV is produced. This product is isomeric with 6-methoxy-9-methyl rhetsinine[2] (V), obtained upon mild basic hydrolysis of hortiamine methiodide (II).



IV



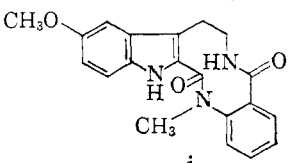
V

The structure of the methylation product IV was confirmed through synthesis. 5-Methoxytryptamine-2-carboxylic acid[8] (VI) reacts with N-methylisatoic anhydride (VII) to produce the *o*-methylaminobenzoyl derivative[9] VIII. Methylation of VIII with methyl iodide at 100° yields the

(1) Presented, in part, at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April, 1958.

(2) I. J. Pachter, R. F. Raffauf, G. E. Ullyot and O. Ribeiro, THIS JOURNAL, **82**, 5187 (1960).

(3) At the outset of our studies we considered structure III and also the ten-membered ring diamide structure i for 6-methoxyrhetsinine. The latter was considered improbable when a Courtauld model of i showed that if the methoxyindole moiety and its attached carbonyl



i

group are coplanar, the remainder of the molecule must stand in an essentially right angle relationship to the plane. A compound of structure i should therefore have an absorption maximum located at a wave length similar to that of 6-methoxy-1-oxo-1,2,3,4-tetrahydropyrid-[3,4-b]indole (XIII, λ_{max}^{EtOH} 306 mμ). Instead, 6-methoxyrhetsinine actually displays an ultraviolet maximum at 318 mμ, which is almost identical in location to that of the 2-benzoyl derivative of XIII[2] (λ_{max}^{EtOH} 319 mμ), a model for structure III.

The data presented in the present paper lead us to reject structure i on chemical grounds.

(4) A. Chatterjee, S. Bose and C. Ghosh, *Tetrahedron*, **7**, 257 (1959).

(5) I. J. Pachter and G. Suld, *J. Org. Chem.*, **25**, 1680 (1960).

(6) K. W. Gopinath, T. R. Govindachari and U. R. Rao, *Tetrahedron*, **8**, 293 (1960).

(7) Prof. T. Ohta of the Tokyo College of Pharmacy, Tokyo, Japan, kindly called our attention to the results of the studies of Dr. Nakazato and co-workers of the Kobe Women's College of Pharmacy which were presented at the 13th General Meeting of the Pharmaceutical Society of Japan, Tokyo, Japan, April, 1960.

(8) R. A. Abramovitch and D. Shapiro, *J. Chem. Soc.*, 4589 (1956).

(9) A reaction similar to this was previously described by Y. Asahina and T. Ohta, *J. Pharm. Soc. Japan*, **49**, 1025 (1929); *C. A.*, **24**, 1386 (1930).