[CONTRIBUTION FROM THE KETTERING-MEYER LABORATORY,[1] SOUTHERN RESEARCH INSTITUTE]

Synthesis of Potential Anticancer Agents. XII. 9-Alkyl-6-substituted-purines[2]

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The 9-*n*-butyl, 9-cyclopentyl and 9-cyclohexyl analogs of purine, adenine and 6-mercaptopurine have been prepared from the corresponding 9-alkyl-6-chloropurines. The chloropurines and the hypoxanthine analogs were prepared by the reaction of the appropriate 4-alkylamino-5-amino-6-chloropyrimidines with diethoxymethyl acetate and formic acid, respectively.

Recently 6-chloro-9-methylpurine was shown to possess the same order of activity against Adenocarcinoma 755 in C 57 black mice as 6-chloropurine.[3] This result was somewhat surprising since this compound cannot be ribosidated *in vivo* without prior removal of the methyl group. However, enzymatic demethylations are common. Since removal of an ethyl group *in vivo* might be less likely to occur, a series of 9-ethyl-6-substituted purines was prepared in this Laboratory[4] for testing. Of these compounds 6-chloro-9-ethylpurine and 9-ethyl-6-mercaptopurine have both shown the same order of activity against Adenocarcinoma 755 as the parent purines.[5] The effectiveness of these two compounds gave impetus to an investigation of the effect of other 9-alkyl groups on the anticancer activity of certain purines. This study should contribute to a better understanding of their mode of action.

The *n*-butyl, cyclopentyl and cyclohexyl groups were chosen as 9-substituents to be investigated. These groups should and do alter the properties of the parent purines markedly.[4] In addition, the steric relationship of the cyclopentyl group to ribose in the furanose form (as it occurs in the natural nucleosides) is obvious.

It was decided to prepare these 9-alkyl derivatives of some of the naturally occurring purines (adenine, hypoxanthine and purine) and of two purines which show marked anticancer activity (6-chloropurine and 6-mercaptopurine).

The necessary chloropurines were prepared from the 4-alkylamino-5-amino-6-chloropyrimidines, which in turn were prepared from 5-amino-4,6-dichloropyrimidine. The general procedures for both these steps have been described.[4] In early experiments ethanolic solutions of the amines were allowed to react with the 5-amino-4,6-dichloropyrimidine in a bomb, but later these reactions were carried out more conveniently on a large scale in refluxing *n*-butyl alcohol.

A study of the conditions employed in the cyclization of the 4-alkylamino-5-amino-6-chloropyrimidines to the corresponding purines was made to determine the relative efficiencies of (1) a mixture of ethyl orthoformate and acetic anhydride, (2) diethoxymethyl acetate in ethyl orthoformate and (3) pure diethoxymethyl acetate. Previously it was found that pure diethoxymethyl acetate or diethoxymethyl acetate in ethyl orthoformate were both superior to the ethyl orthoformate–acetic anhydride mixture for the preparation of 2,6-dichloropurine.[2]

The cyclization of 5-amino-4-*n*-butylamino-6-chloropyrimidine was carried out in pure diethoxymethyl acetate and in a mixture of ethyl orthoformate and acetic anhydride. The ethyl orthoformate–acetic anhydride mixture gave a lower yield of purine (contaminated with 9-*n*-butyl-6-hydroxypurine), and the reaction rate was roughly half that of the diethoxymethyl acetate reaction, judged by the ultraviolet spectra of aliquots from the reaction mixture.

The same type of comparison described above was made of the cyclization of 5-amino-6-chloro-4-cyclopentylaminopyrimidine in pure diethoxymethyl acetate and with three equivalents of diethoxymethyl acetate in ethyl orthoformate. Reducing the amount of diethoxymethyl acetate slowed the reaction to such an extent that an additional amount of this material had to be added to cause the reaction to go to completion. Even then the yield of pure 6-chloro-9-cyclopentylpurine was lower than that obtained when pure diethoxymethyl acetate was employed. The low yield of the 6-chloro-9-cyclopentylpurine (30%) compared to that of the 9-cyclohexyl (62%) and 9-*n*-butyl (87%) compounds resulted from difficulties in the purification procedure and undoubtedly could be improved.

It was also found that the use of a lower reaction temperature (100°) than originally employed with pure diethoxymethyl acetate[4] resulted in fewer side reactions and, therefore, the product obtained was easier to purify.

All these results show that pure diethoxymethyl acetate is superior for these cyclizations and substantiate the contention that this compound is the true reactant in ethyl orthoformate–acetic anhydride ring closures.

The 9-alkyl-6-chloropurines were converted to the corresponding purines, adenines and 6-mercaptopurines by known procedures.[4,6]

It is interesting that these chloropurines failed to react with thiourea in boiling ethanol, but did react smoothly at the higher temperature afforded by boiling *n*-propyl alcohol. The nature of the 9-alkyl group apparently affects the reactivity of the 6-chlorine atom, since 6-chloro-9-ethylpurine reacted readily with thiourea in ethanol.[4]

The hypoxanthine analogs were prepared by the cyclization of the 4-alkylamino-5-amino-6-chloro-

(1) Affiliated with Sloan-Kettering Institute for Cancer Research. This work was supported by funds from the C. F. Kettering Foundation.

(2) Part XI, J. A. Montgomery and L. B. Holum, THIS JOURNAL, **80**, 404 (1958).

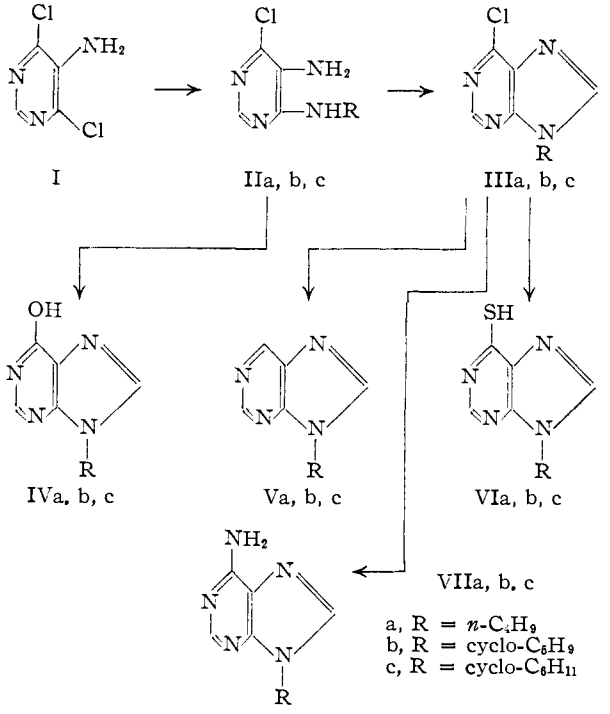
(3) H. E. Skipper, J. R. Thomson and R. K. Robins, unpublished data.

(4) J. A. Montgomery and Carroll Temple, Jr., THIS JOURNAL, **79**, 5238 (1957).

(5) H. E. Skipper and J. R. Thomson, unpublished data.

(6) (a) R. K. Robins and H. H. Lin, THIS JOURNAL, **79**, 490 (1957) (b) A. Bendich, P. J. Russell and J. J. Fox, *ibid.*, **73**, 6073 (1954).

pyrimidine with formic acid, which causes concomitant hydrolysis of the 6-chlorine atom.[6a] This



procedure is more convenient than hydrolysis of the 9-alkyl-6-chloropurines.[4]

The yields, recrystallization solvents, melting points and elemental analyses of the purines prepared are listed in Table I. Typical examples of the procedures employed are given in the Experimental section.

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Experimental[7]

5-Amino-4-*n*-butylamino-6-chloropyrimidine (IIa).—A solution of 5-amino-4,6-dichloropyrimidine[8] (10 g., 61 mmoles) and *n*-butylamine (20 g., 274 mmoles) in ethanol (100 ml.) was heated in a stainless steel bomb[9] at 125–130° for six hours. The solution was evaporated to dryness, the residue triturated with ether (300 ml.), and the insoluble solid (*n*-butylamine hydrochloride) removed by filtration. The ether was removed and the residual oil distilled *in vacuo* giving a yellow solid; yield 9.0 g. (74%), b.p. 128° (0.07–0.09 mm.). A carbon and hydrogen analysis indicated that this material was 98% pure.

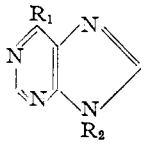
A small sample of the above solid was recrystallized from Skellysolve C; m.p. 78–79°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 276 (shoulder) (8.34), 301 (11.5); 0.1 *N* NaOH, 263 (8.96), 289 (9.0).

Anal. Calcd. for $C_8H_{13}ClN_4$: C, 47.88; H, 6.48; N, 27.93. Found: C, 47.72; H, 6.63; N, 27.96.

5-Amino-6-chloro-4-cyclopentylaminopyrimidine (IIb).—A solution of 5-amino-4,6-dichloropyrimidine (10 g., 61 mmoles) and cyclopentylamine[10] (24 g., 282 mmoles) in methanol (80 ml.) was heated in a stainless steel bomb at 125–130° for six hours. The solution was evaporated to dryness and the residue sublimed at 135° (0.01–0.05 mm.); yield 7.33 g. (56.5%). A carbon and hydrogen analysis indicated that this material was 97% pure.

A small sample was recrystallized from water and dried over P_2O_5 *in vacuo* at 80°; m.p. 140–142°; λ_{max} in mμ ($\epsilon \times$

TABLE I



R_2 = *n*-C_4H_9 R_1 =	Yield, %	Recrystn. solvent[a]	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H	64	..[b]	~30	61.34	60.96	6.86	6.80	31.80	31.89
Cl	87	..	....[c]	51.31	51.21	5.22	5.71	26.55	26.43
NH_2	88	A	138–139	56.52	56.48	6.85	6.74	36.63	36.44
OH	51	B	259–260	56.23	56.01	6.29	6.09	29.15	29.21
SH	70.5	C	311–312	51.90	51.77	5.80	5.43	26.90	26.73
R_2 = cyclo-C_5H_9, R_1 =									
H	65	..[d]	80–81	63.81	63.39	6.41	6.41	29.77	29.94
Cl	30	B	96	53.93	53.86	4.94	5.37	25.15	25.16
NH_2	91	A + D	156	59.09	58.82	6.45	6.47	34.46	34.53
OH	51	B	230	58.80	58.90	5.92	5.89	27.44	27.46
SH	81	C	>300	54.54	54.62	5.49	5.66	25.44	25.15
R_2 = cyclo-C_6H_{11}, R_1 =									
H	85	..[d]	95–96	65.32	65.02	6.98	7.10	27.70	27.85
Cl	57	D	114	55.75	56.06	5.49	5.68	23.65	23.45
NH_2	62	D	200	60.80	61.03	6.96	6.95	32.24	32.16
OH	83.5	B	273–275	60.53	60.45	6.47	6.49	25.67	25.49
SH	73	E	350	56.40	56.12	6.02	6.21	23.92	23.64

[a] A, benzene; B, water; C, methyl isobutyl ketone; D, Skellysolve C; E, precipitated from dil. NaOH with acetic acid. [b] Distilled; b.p. 90–91° (0.05 mm.). [c] Liquid, b.p. 142° (0.2 mm.). [d] Sublimed *in vacuo*.

The ultraviolet spectra of all these compounds are practically identical with those of the corresponding 9-methyl-[6a] and 9-ethylpurines.[4] The infrared spectra are also very similar to those of the 9-ethylpurines except for the greater intensity of the bands in 3000–2800 cm.$^{-1}$ region due to aliphatic CH.

(7) Melting points below 260° were determined on a Kofler Heizbank; those above 260° were determined in a capillary tube in aluminum block and are uncorrected.

(8) D. J. Brown, *J. Appl. Chem.*, **4**, 72 (1954).

(9) Parr Instrument Co.

(10) J. R. Corrigan, M. J. Sullivan, H. W. Bishop and A. W. Ruddy, THIS JOURNAL, **75**, 6259 (1953).

10^{-3}): 0.1 *N* HCl, 277 (shoulder) (8.8), 305 (12.3); 0.1 *N* NaOH, 264.5 (9.4), 290 (9.66).

Anal. Calcd. for $C_9H_{13}ClN_4$: C, 50.80; H, 6.12; N, 26.30. Found: C, 50.98; H, 6.48; N, 26.00.

5-Amino-6-chloro-4-cyclohexylaminopyrimidine (IIc).—A solution of 5-amino-4,6-dichloropyrimidine (10 g., 61 mmoles) and cyclohexylamine (30 g., 305 mmoles) in ethanol (70 ml.) was heated in a stainless steel bomb[10] at 130° for six hours. The solution was evaporated to dryness, the residue triturated with ether (500 ml.), and the insoluble solid (cyclohexylamine hydrochloride) removed by filtration. The ether was removed and the residue extracted by decantation with boiling water (6 × 200 ml.). The combined, cooled extracts yielded 2.15 g. of a light yellow solid, m.p. 138°.

Anal. Calcd. for $C_{10}H_{15}ClN_4 \cdot H_2O$: C, 49.00; H, 6.95; N, 22.85. Found: C, 49.27; H, 6.89; N, 22.57.

The sample (2.0 g.) was dried *in vacuo* over P_2O_5 at 100° for four hours; yield 1.87 g., m.p. 139°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 275 (shoulder) (8.78), 304 (12.3); 0.1 *N* NaOH, 262.5 (9.65), 288 (9.45).

Anal. Calcd. for $C_{10}H_{15}ClN_4$: C, 52.90; H, 6.62; N, 24.70. Found: C, 53.26; H, 6.71; N, 24.62.

The light brown residue from the above aqueous extraction was dried *in vacuo* over P_2O_5 at 100° for four hours; yield 7.68 g., m.p. 138°. The ultraviolet spectrum of this material was practically identical with those of the analytical sample above. The total yield of 5-amino-6-chloro-4-cyclohexylaminopyrimidine was 9.83 g. (71%).

9-*n*-Butyl-6-chloropurine (IIIa).—A solution of 5-amino-4-*n*-butylamino-6-chloropyrimidine (8.1 g.) in diethoxymethyl acetate[11] (75 ml.) was heated at 100° for 1.5 hours and then evaporated to a small volume *in vacuo*. Distillation of the residue under reduced pressure gave a light yellow liquid; yield 7.42 g., b.p. 142° (0.2 mm.).

9-Cyclohexyl-6-hydroxypurine (IVc).—A solution of 5-amino-6-chloro-4-cyclohexylaminopyrimidine (1.0 g.) in anhydrous formic acid (10 ml.) was refluxed for 5 hours, evaporated to a small volume, diluted with water (10 ml.) and neutralized to *p*H 8 with concd. ammonium hydroxide. The mixture was cooled, and the solid which deposited was collected by filtration and dried over P_2O_5; yield 800 mg., m.p. 268–270°. A carbon and hydrogen analysis indicated that this material was 98% pure. Recrystallization of a small sample from water raised the melting point to 273–275°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 249 (11.7); 0.1 *N* NaOH, 254 (13.1).

9-*n*-Butylpurine (Va).—A solution of 9-*n*-butyl-6-chloropurine (3.47 g., 16.5 mmoles) in a 1:1 mixture of ethanol–water (100 ml.) was hydrogenated over a 5% Pd/C catalyst (2 g.) in the presence of magnesium oxide (2 g.). After the mixture had absorbed 420 ml. of hydrogen (theoretical 411 ml.), the catalyst and excess magnesium oxide were removed by filtration and washed with hot ethanol (50 ml.). To the combined filtrates was added 50 ml. of a 5% sodium carbonate solution and the whole evaporated to dryness *in vacuo*. The residue was extracted with ether (3 × 75 ml.) and the ether removed at reduced pressure. Distillation of the residue *in vacuo* gave a colorless liquid; yield 2.45 g. (84.5%). A carbon and hydrogen analysis indicated that this material was 98% pure. A second distillation was necessary to obtain the analytical sample; yield 1.84 g.; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 263 (5.6); 0.1 *N* NaOH, 264 (7.6).

9-Cyclopentyl-6-mercaptopurine (VIb).—A solution of 6-chloro-9-cyclopentylpurine (500 mg., 2.25 mmoles) and thiourea (170 mg., 2.25 mmoles) in *n*-propyl alcohol (15 ml.) was refluxed for 15 minutes. The solid that deposited was collected by filtration, dissolved in 1 *N* NaOH (10 ml.), and the solution acidified with acetic acid. The white precipitate was removed by filtration, washed with water, and dried *in vacuo* over P_2O_5 at 80°; yield 400 mg.

A small sample was recrystallized from methyl isobutyl ketone; m.p. >300°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 224 (9.35), 325 (19.1); 0.1 *N* NaOH, 232 (14.4), 311 (22.4).

6-Amino-9-cyclohexylpurine (VIIc).—A solution of 6-chloro-9-cyclohexylpurine (1.5 g.) in ethanolic ammonia (50 ml. saturated at 0°) was heated in a bomb for 15 hours at 105–110°. The solution was evaporated to dryness *in vacuo*, and the residue boiled in benzene (50 ml.). A brown, insoluble material was removed from the benzene solution by filtration, and the filtrate allowed to cool. The solid which deposited was collected by filtration and dried *in vacuo* over P_2O_5; yield 700 mg., m.p. 199–200°. Recrystallization from Skellysolve C did not raise the melting point; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 260 (14.5); 0.1 *N* NaOH, 262 (14.9).

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(11) H. W. Post and E. R. Erickson, *J. Org. Chem.*, **2**, 260 (1937).

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

The *in vivo* Hydroxylation of 1-Ethynylcyclohexyl Carbamate[1,2]

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1-Ethynylcyclohexyl carbamate-C^{14} (ethinamate-C^{14}) has been prepared and its metabolism studied in rats. Hydrolytic cleavage of the carbamate grouping was found to be a minor catabolic route, the major route being hydroxylation to hydroxyethynylcyclohexyl carbamate (hydroxyethinamate). Hydroxyethinamate is eliminated in urine both in the unbound form and as the β-glucuronide. Hydroxyethinamate was isolated from human urine and its identity conclusively shown by physical properties and by derivative formation. No evidence was found that the acetylene group was altered by the body. The significance of these findings is discussed.

Introduction

The metabolic conversion of the acetylenic grouping in animal organisms has been little studied. Hoppe-Seyler[3] early reported the conversion of *o*-nitrophenylpropiolic acid to indoxyl. Bohm[4] later reported that *o*-nitrophenylacetylene is likewise metabolized to indoxyl. These reports together with the observation by El Masry, Smith and Williams[5] of the slow conversion of phenylacetylene to phenylacetic acid by rabbits appear to represent the major investigations in which metabolic products of acetylenic compounds have been clearly identified.

The recently introduced hypnotic drug ethinamate (1-ethynylcyclohexyl carbamate, I) has been reported by Langecker, Schumann and Junkmann[6] and by Swanson, Anderson and Gibson[7] to be

(1) Eli Lilly and Company Trade-mark, "Valmid" (Ethinamate, Lilly).

(2) Preliminary Report, Medicinal Chemistry Division, A.C.S. 130th Meeting, Atlantic City, New Jersey, September, 1956.

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(4) F. Bohm, *ibid.*, **261**, 35 (1939).

(5) A. M. El Masry, J. N. Smith and R. T. Williams, *Biochem. J.*, **65**, No. 2, 10P (1957).

(6) H. Langecker, H. J. Schumann and K. Junkmann, *Arch. exptl. Pathol. Pharmakol.*, **219**, 130 (1953).

(7) E. E. Swanson, R. C. Anderson and W. R. Gibson, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 40 (1956)