Synthesis of Potential Antineoplastic Agents. XXXI. 9-Alkyl-9*H*-Purines[1]

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Some time ago in this laboratory a number of 9-alkyl-9*H*-purines were prepared for evaluation against experimental animal tumors.[2] Most of the 9-alkyl derivatives of 6-chloropurine and 6-mercaptopurine did indeed show marked inhibitory action against Adenocarcinoma 755[3] and some of them showed a lower order of activity against Sarcoma 180 and Leukemia L1210. More recently 9-ethyl-6-mercaptopurine has shown activity against chronic adult human leukemias.[4]

One of the major stumbling blocks to the successful treatment of human cancer today is the development of resistance[5] to drugs such as 6-mercaptopurine. In one approach to the problem, we have evaluated a number of compounds[6] for cytotoxic effects against two sublines of human epidermal carcinoma (H. Ep-2) that is resistant to the action of 6-mercaptopurine.[7] The 9-alkyl-9*H*-purines have been found to be the most effective inhibitors of these cell lines.[6]

In an effort to correlate the nature of the 9-substituent with the inhibitory activity of these compounds and to pinpoint the most effective member of this series, a number of new 9-substituted 9*H*-purines have now been synthesized for evaluation. The required 4-alkylamino-5-amino-6-chloropyrimidines (I) were prepared from 5-amino-4,6-dichloropyrimidine (II) by convenient modifications of the usual procedure.[2,8] Instead of bomb reactions used previously

(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740. Part XXX, J. A. Montgomery, K. Hewson, and C. Temple, Jr., *J. Med. Pharm. Chem.*, **5**, 15 (1962).

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with low boiling amines, a five-fold excess of amine in refluxing propanol was employed for the smooth and rapid amination of II. Furthermore, the higher boiling amines produced monoaminated pyrimidines when employed in excess as both reactant and solvent at a temperature of about 100°. None of the 5-amino-4-(substituted-amino)-pyrimidines (I) prepared were purified since the crude pyrimidines gave satisfactory yields in later conversions.

Although the cyclization of compounds similar to I with ethyl orthoformate–acetic anhydride or diethoxymethyl acetate generally produces good yields of 9-substituted 6-chloropurines (III), only fair yields are obtained in some cases.[2] The lower yields of III have been attributed to the detrimental but apparently required reaction temperatures which produce contaminants that are difficult to remove from the product.[2]

The chloropurines described herein (Table I) were obtained in excellent yields by simply stirring solutions or suspensions of I in ethyl orthoformate overnight at room temperature in the presence of a slight excess of concentrated hydrochloric acid. The increase in yields of III over those of similar compounds prepared by other methods is probably due to the conditions of the reaction.[9] The isolation of the three 6-chloro-9-propyl-9*H*-purines from the reaction mixture as hydrochlorides is undoubtedly due to their insolubility rather than to greater basicity of the purines.

The hypoxanthine, adenine and 6-mercaptopurine analogs listed in Table I were prepared by published procedures; the hypoxanthines from I and formic acid,[2,10] and the adenines and 6-mercaptopurines from III and the appropriate nucleophile.[2] In addition, 6-chloro-9-propyl-9*H*-purine was converted in refluxing formic acid to 9-propylhypoxanthine, but this route offers no advantage if 5-amino-6-chloro-4-propylaminopyrimidine is available.

Typical ultraviolet and infrared spectra of the 6,9-disubstituted purines have been reported elsewhere and are not listed here.[2]

Cell Culture Cytotoxicity.—Although none of the compounds described in this paper showed a higher degree of inhibition of the 6-mercaptopurine-resistant H.Ep-2 cells than the cyclopentyl and cyclohexyl derivatives previously reported,[6] the normal hexyl derivatives were the most effective inhibitors of 6-mercaptopurine-resistant Adenocarcinoma 755 cells in culture.[11] Further studies on the effect

(9) In the present work, the application of moderate heat for a short period in an attempted preparation of 6-chloro-9-hexyl-9*H*-purine led to the complete hydrolysis of the chloro group and the production of 9-hexylhypoxanthine.

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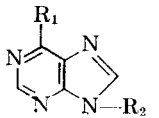
(11) Dr. G. G. Kelley, personal communication.

TABLE I



R_1 =	Recrystn. solvent[a]	Yield, %	M.p. or b.p., °C.[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
				R_2 = Propyl					
Cl[c]	A	92[d]	193–195[c]	41.22[e]	41.63	4.32[e]	4.67	24.03[e]	24.38
OH	A[f]	35[d,l]	259–260	53.92	54.06	5.65	5.59	31.44	31.45
NH_2	A	62	173	54.22	54.29	6.25	6.24	39.53	39.68
SH	B	66	321–323	49.45	49.48	5.20	5.16	28.43	29.04
				R_2 = Isopropyl					
Cl[c]	..[g]	83[d]	153[c,i]	41.22[g]	41.23	4.32[g]	4.17	24.03[g]	24.22
OH	A[f]	62[d]	222	53.92	53.58	5.65	5.59	31.44	31.46
NH_2	C	28	235–236[i]	44.96[j]	44.79	5.66[j]	5.32	32.77[j]	32.68
SH	B	79	335–338	49.45	49.32	5.20	5.03	28.83	29.04
				R_2 = Cyclopropyl					
Cl[c]	A	91[d]	124[c]	41.57[h]	41.54	3.49[h]	3.39	24.25[h]	24.08
OH	A[f]	46[d]	291–293	54.53	54.56	4.58	4.86	31.80	31.73
NH_2	E	38	256–258[l]	45.39[k]	45.35	4.76[k]	4.62	33.09[k]	33.17
SH	F	96	>345	49.99	50.01	4.20	4.31	29.15	28.97
				R_2 = Pentyl					
Cl	..	79[d]	152–156 (0.7 mm)	53.45	53.76	5.83	5.91	24.93	24.76
OH	B	87[d]	262	58.24	58.13	6.84	6.73	27.17	27.37
NH_2	D[f]	77	132–135	58.51	58.75	7.37	7.30	34.12	33.88
SH	B	77	309–312	54.03	54.31	6.35	6.31	25.21	24.98

			R_2 = Hexyl						
Cl	..	75[d]	160 (0.2 mm)	55.35	55.79	6.34	6.35	23.47	23.23
OH	B	55[d]	254	59.97	59.83	7.32	7.53	25.45	25.45
NH_2	D	73	146	60.24	60.13	7.81	8.05	31.94	32.24
SH	B	64	304–306	55.91	55.95	6.82	6.80	23.71	23.75
			R_2 = Heptyl						
Cl	..	48[d]	164–167 (0.3 mm)	57.01	57.06	6.78	6.92	22.17	21.82
OH	B	61[d]	249	61.52	61.41	7.74	7.44	23.92	23.76
NH_2	D	83	127	61.77	61.64	8.21	7.97	30.02	30.07
SH	C	37	292–295	57.56	57.48	7.25	7.15	22.37	22.27
			R_2 = Cycloheptyl						
Cl	G	86[d]	103	57.49	57.54	6.03	6.07	22.35	22.14
OH	A[f]	55[d]	250–251	62.04	61.97	6.94	6.95	24.12	24.05
NH_2	A	82	177	62.31	62.29	7.41	7.33	30.28	30.56
SH	F	91	>345	58.02	57.92	6.50	6.49	22.56	22.43
			R_2 = Octyl						
Cl	..	81[d]	168–171 (0.5 mm)	58.52	58.34	7.20	7.49	21.00	20.80
OH	B	72[d]	244	62.88	62.78	8.11	8.19	22.56	22.72
NH_2	H	63	131	63.13	63.26	8.56	8.45	28.32	28.14
SH	C	66	287	59.05	59.00	7.63	7.64	21.19	21.00

[a] A, acetonitrile; B, precipitated from dil. sodium hydroxide with glacial acetic acid; C, ethanol; D, benzene; E, trituration with hot benzene; F, trituration with propanol; G, ethyl acetate; H, Skellysolve C. [b] Unless otherwise indicated, melting points below 260° were determined on a Kofler Heizbank and are corrected; melting points above 260° were determined in a capillary tube and are uncorrected. [c] Isolated and purified as the hydrochloride. [d] Based on amount of 5-amino-4,6-dichloropyrimidine employed. [e] $C_8H_9ClN_4 \cdot HCl$: Calcd., Cl, 30.43; found, 30.22. [f] Soxhlet extraction. [g] Pure HCl deposited from reaction mixture; $C_8H_9ClN_4 \cdot HCl$: Calcd., Cl, 30.43; found, 30.54. [h] Calcd. for $C_8H_7ClN_4 \cdot HCl$: Cl, 30.69; found, 30.30. [i] Capillary. [j] Calcd. for $C_8H_{11}N_5 \cdot HCl$. [k] Calcd. for $C_8H_9N_5 \cdot HCl$. [l] Obtained in 42% yield from 6-chloro-9-*n*-propylpurine in refluxing formic acid.

of the various 9-alkyl-9*H*-purine in other 6-mercaptopurine-resistant biological systems is in progress.

Experimental

Amination of 5-Amino-4,6-dichloropyrimidine.—A solution of 5-amino-4,6-dichloropyrimidine (50 g.), either in a five-fold excess of the amine or in propanol (*ca.* 500 ml.) containing a five-fold excess of the low boiling amine, was heated at 100–110° in an oil bath for 2 hr. in an atmosphere of dry nitrogen. The residue obtained by evaporation of the volatiles *in vacuo* was treated by one of the following methods: (A) The residues containing the 4-propyl-, 4-pentyl-, 4-hexyl-, 4-heptyl- and 4-octylaminopyrimidines were extracted with ether (4 × 1000 ml.) and the combined extracts evaporated to dryness to yield the crude pyrimidines. Further removal of amine from the 4-octylaminopyrimidine was effected by neutralization of an aqueous suspension of the crude pyrimidine with dilute hydrochloric acid. The 4-propylaminopyrimidine required a second ether extraction to remove excess amine hydrochloride. (B) The residues containing the 4-isopropyl- and 4-cyclopropylaminopyrimidines were triturated with water (1000 ml.) and the crude pyrimidines collected by filtration. The 4-cycloheptylaminopyrimidine was isolated by ether extraction of a neutralized aqueous suspension of the residue.

Cyclization of 5-Amino-6-chloro-4-(substituted-amino)pyrimidines.—To 1 mmole of the pyrimidine (I) dissolved in triethyl orthoformate (4 ml.) was added concd. hydrochloric acid (1.3 mmoles) in small increments with stirring. In some cases the hydrochloride of the pyrimidine precipitated. The solution or suspension was stirred overnight at room temperature to effect conversion to the 6-chloropurine, which was isolated by one of the following methods: (A) The suspended hydrochloride of the 6-chloropurine was collected by filtration and recrystallized from the appropriate solvent (Table I). (B) The clear solution was concentrated under reduced pressure in a 60° oil-bath and the resulting gum of 6-chloropurine was washed with Skellysolve C and distilled under high vacuum (Table I). Solid 6-chloro-9-cycloheptyl-9*H*-purine, obtained by washing the gum with Skellysolve C, was purified by ether trituration or recrystallization from ethyl acetate.

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