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Aminomethylation and Hydroxymethylation of 6-Mercaptopublne and 6-Alkylthiopubines

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AMINOMETHYLATION AND HYDROXYMETHYLATION OF 6-MERCAPTOPURINE AND 6-ALKYLTHIOPURINES

by

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INTRODUCTION

The Mannich reaction and hydroxymethylation were used to prepare analogs of 6-mercaptopurine as possible antimetabolites. Physical and chemical methods were used to elucidate the structures of the compounds prepared.

HISTORICAL

The reaction between a secondary amine, formaldehyde, and a compound having an acidic hydrogen, the Mannich reaction, has been extensively studied in many different systems.[1, 2] However, in the purine system very little work has been done. Burckhalter and Dill[3] reacted theophylline with formaldehyde and various secondary amines to get 7-α-dialkylamino derivatives of caffeine. The only one of these products which was stable enough for one recrystallization, though, was the 7-α-morpholino-caffeine.

Hydroxymethylation of caffeine was studied by H. Bredereck, E. Siegel and B. Fohlisch.[4] This purine hydroxymethylated in the 8 position. Reaction of the 8-hydroxymethyl derivative with thionyl chloride gave the expected 8-chloromethyl compound. This compound was then reacted with secondary amines to give 8-dialkylamino derivatives.

Hydroxymethylation has been accomplished with formaldehyde on 2', 3'-O-isopropylidene uridine, 2', 3'-O-isopropylidene adenosine, 2', 3'-O-isopropylidene cytidine and 2', 3'-O-isopropylidene guanosine by K. H. Scheit.[5] The reaction of 6-azuaracil and 6-azathymine with formaldehyde as well as some other reactive aldehydes was studied by M. Prystas and F. Sorm.[6]

In a study done by G. B. Bachman and L. V. Heisey[7]

it was found that when benzimidazole and pyrrole are mixed in the presence of one equivalent of formaldehyde, only 1-hydroxymethylbenzimidazole was obtained.

The hydrogen attached to nitrogen in many amides is acidic enough to take part in the hydroxymethylation reaction. H. Hellman[8] gives a review of this particular system.

EXPERIMENTAL

General

All melting points are expressed in degrees centigrade and are corrected. The elemental analyses were performed by Galbraith Microanalytical Laboratories. The analytical samples were dried at least twenty-four hours *in vacuo* over anhydrous calcium chloride before shipment. Infrared spectra were run in potassium bromide pellets on a Beckman IR-8 instrument. The ultraviolet absorption spectra were taken on a Cary Model 14 Spectrophotometer. The 6-alkylthiopurines and the 6-mercaptopurine, monohydrate were obtained from the Cancer Chemotherapy National Service Center and were used as obtained without further purification. The notation, ϵ_M, used throughout the Experimental refers to the molar absorptivity.

6-METHYLTHIO-9-HYDROXYMETHYLPURINE (I). To 10 ml. of 37% formalin was added 1.66 g. (.01 mole) of 6-methylthiopurine. After the material went into solution a precipitate was formed. The mixture was stirred mechanically for ten minutes and allowed to stand at room temperature for one hour. The solid was separated by suction filtration, washed with cold water and air dried overnight. The yield was 1.62 g. (83%). Recrystallization from ethyl acetate gave a crystalline material with m.p. 158-161°. Descending paper chromatography using Whatman no. 1

paper with water-saturated butanol as carrier in an ammonia atmosphere gave one spot. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 288 mμ (ϵ_M = 1.87 x 10^4).

Analytical calculated for $C_7H_8N_4OS$: C, 42.84%; H, 4.11%; N, 28.55%. Found: C, 42.85%; H, 4.04%; N, 28.22%.

6-ETHYLTHIO-9-HYDROXYMETHYLPURINE (II). To 10 ml. of 37% formalin was added 1.80 g. (.01 mole) of 6-ethylthiopurine. After all the material had been dissolved the reaction mixture was heated to 35° and 0.62 g. (.005 mole) of sodium carbonate, monohydrate was added. To get the sodium carbonate, monohydrate into solution required the addition of 7 ml. of water. The solution was allowed to stand overnight. The solid which separated was collected by suction filtration and dried. The yield was 1.45 g. (69%). Recrystallization from ethyl acetate gave a crystalline material with m.p. 122-123°. Descending paper chromatography using Whatman no. 1 paper with water-saturated butanol as carrier in an ammonia atmosphere gave one spot. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 289 mμ (ϵ_M = 1.72 x 10^4).

Analytical calculated for $C_8H_{10}N_4OS$: C, 45.70%; H, 4.79%; N, 26.65%. Found: C, 45.81%; H, 4.92%; N, 26.49%.

6-(n-PROPYLTHIO)-9-HYDROXYMETHYLPURINE (III). To a solution of 3.92 g. (.02 mole) of 6-(n-propylthio)-purine in 25 ml. of 37% formalin was added 1.66 g. (.02 mole) of sodium bicarbonate. The solid material which immediately separated was redissolved by adding 20 ml. of methanol and

gently heated. The resulting solution was filtered and crystallization effected by cooling in an ice bath. The yield was 4.0 g. (88%). Recrystallization from ethyl acetate gave a crystalline material with m.p. 123-125.5°. Descending paper chromatography using Whatman no. 1 paper with water-saturated butanol as carrier in an ammonia atmosphere gave one spot. Thin layer chromatography using Silica Gel G with methyl alcohol as carrier gave one sharp spot. With ethyl acetate as carrier, again, only one sharp spot was obtained. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 291 mμ (ϵ_M = 1.8 x 10^4).

Analytical calculated for $C_9H_{12}N_4OS$: C, 48.20%; H, 5.39%; N, 24.98%. Found: C, 48.41%; H, 5.43%; N, 25.11%.

6-(n-BUTYLTHIO)-9-HYDROXYMETHYLPURINE (IV). To a solution of 6.24 g. (.03 mole) of 6-(n-butylthio)-purine in 25 ml. of 37% formalin was added 2.52 g. (.03 mole) of sodium bicarbonate. The mixture was stirred for one hour and then allowed to stand overnight, whereupon some crystals began to form. The mixture was cooled in an ice bath and the oil which separated was redissolved by adding methanol and warming to 40°. Cooling in an ice-salt mixture brought about crystallization. The solid was collected by suction filtration and washed with water. Addition of water to the supernatant liquid gave a second crop of crystals. The combined crystalline product was dissolved in a small amount of ethyl acetate at 45° and crystallization occurred at room temperature. The yield

was 5.5 g. (78%). Recrystallization from ethyl ether gave a crystalline material with m.p. 82-84°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 291 mμ ($\epsilon_M = 1.92 \times 10^4$).

Analytical calculated for $C_{10}H_{14}N_4OS$: C, 50.40%; H, 5.92%; N, 23.51%. Found: C, 50.44%; H, 5.78%; N, 23.47%.

6-(n-PENTYLTHIO)-9-HYDROXYMETHYLPURINE (V). To a mixture of 1.5 g. (.0067 mole) of 6-(n-pentylthio)-purine in 10 ml. of 37% formalin was added 0.42 g. (.0034 mole) of sodium carbonate, monohydrate which had been dissolved in a minimum amount of water. This mixture was stirred for ten minutes and then allowed to stand for two hours. The solid was separated by suction filtration and air dried overnight. The yield was 1.6 g. (94%). Recrystallization from ethyl acetate gave a crystalline material with m.p. 110-111° The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 292 mμ ($\epsilon_M = 1.93 \times 10^4$).

Analytical calculated for $C_{11}H_{16}N_4OS$: C, 52.35%; H, 6.39%; N, 22.21%. Found: C, 52.51%; H, 6.55%; N, 22.04%.

6-CYCLOPENTYLTHIO-9-HYDROXYMETHYLPURINE (VI). To a mixture of 2.20 g. (.01 mole) of 6-cyclopentylthiopurine in 10 ml. of 37% formalin was added 0.62 g. (.005 mole) of sodium carbonate, monohydrate which had been dissolved in a minimum amount of water. The mixture was stirred for ten minutes and then allowed to stand overnight. The solid material was separated by suction filtration and air dried. Recrystallization from ethyl acetate yielded 2.4 g. (96%) of crystalline material having a

melting point of 139-140°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 292 mμ (ϵ_M = 1.77 x 10^4).

Analytical calculated for $C_{11}H_{14}N_4OS$: C, 52.78%; H, 5.64%; N, 22.38%. Found: C, 52.98%; H, 5.84%; N, 22.12%.

6-(n-HEXYLTHIO)—9-HYDROXYMETHYLPURINE (VII). To a mixture of 2.35 g. (.01 mole) of 6-(n-hexylthio)-purine in 15 ml. of 37% formalin was added 0.62 g. (.005 mole) of sodium carbonate, monohydrate which had been dissolved in a minimum amount of water. The mixture was stirred for thirty minutes and then allowed to stand overnight. The solid material was separated by suction filtration and air dried. The yield was 2.2 g. (83%). Recrystallization from ligroin (b.p. 85-110°) gave a crystalline material with m.p. 82-84°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 292 mμ (ϵ_M = 1.99 x 10^4).

Analytical calculated for $C_{12}H_{18}N_4OS$: C, 54.11%; H, 6.81%; N, 21.04%. Found: C, 54.32%; H, 7.03%; N, 20.81%.

6-(n-HEPTYLTHIO)-9-HYDROXYMETHYLPURINE (VIII). To a mixture of 2.00 g. (.008 mole) of 6-(n-heptylthio)-purine in 15 ml. of 37% formalin was added 0.5 g. (.004 mole) of sodium carbonate, monohydrate which had been dissolved in a minimum amount of water. The mixture was stirred for thirty minutes and allowed to stand for six hours. The solid was separated by suction filtration and allowed to stand overnight. The yield was 2.0 g. (89%). Recrystallization from ligroin (b.p. 85-110°) gave a crystalline material with m.p. 90-92°. The ultraviolet absorption

spectrum in 95% ethyl alcohol gave λ_{max} = 292 mμ (ϵ_M = 2.08 x 10^4).

Analytical calculated for $C_{13}H_{20}N_4OS$: C, 55.68%; H, 7.19%; N, 19.98%. Found: C, 55.87%; H, 7.27%; N, 20.27%.

6-(n-OCTYLTHIO)-9-HYDROXYMETHYLPURINE (IX). To a mixture of 2.64 g. (.01 mole) of 6-(n-octylthio)-purine in 15 ml. of 37% formalin was added 0.62 g. (.005 mole) of sodium carbonate, monohydrate which had been dissolved in a minimum amount of water. This mixture was stirred for thirty minutes and allowed to stand overnight. The solid material was collected by suction filtration and air dried. The yield was 2.7 g. (92%). Recrystallization from ligroin (b.p. 85-110°) gave a crystalline material with m.p. 76-78°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 291 mμ (ϵ_M = 2.22 x 10^4).

Analytical calculated for $C_{14}H_{22}N_4OS$: C, 57.11%; H, 7.53%; N, 19.05%. Found: C, 57.32%; H, 7.70%; N, 19.01%.

6-(n-DECYLTHIO)-9-HYDROXYMETHYLPURINE (X). To a mixture of 2.92 g. (.01 mole) of 6-(n-decylthio)-purine in 35 ml. of 37% formalin was added 0.62 g. (.005 mole) of sodium carbonate, monohydrate. This mixture was stirred for thirty minutes and allowed to stand for six hours. The solid was separated by suction filtration and the product was air dried overnight. The yield was 2.8 g. (87%). Recrystallization from benzene gave a crystalline material with m.p. 85-88°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 292 mμ (ϵ_M = 1.92 x 10^4).

Analytical calculated for $C_{16}H_{26}N_4OS$: C, 59.59%;

H, 8.13%; N, 17.37%. Found: C, 59.60%; H, 8.18%; N, 17.41%.

9-MORPHOLINOMETHYL-6-PURINETHIOL (XI). A. 6-Mercaptopurine, monohydrate (5.1 g., 0.03 mole) was dissolved in 50 ml. of 0.6 N sodium hydroxide solution. To this solution was added 2.46 g. (.03 mole) of morpholine and the solution stirred for fifteen minutes. A 37% formalin solution, 2.5 g., was now added to the reaction mixture and stirring was continued for eight hours. The reaction mixture was neutralized with acetic acid and the solid collected by suction filtration. The product was air dried overnight. The yield was 6.0 g. (80%). Recrystallization from ethyl alcohol gave a crystalline product with m.p. 230° dec. The ultraviolet absorption spectrum gave λ_{max} = 328 mμ ($\epsilon_M = 2.14 \times 10^4$).

Analytical calculated for $C_{10}H_{13}N_5OS$: C, 47.79%; H, 5.21%; N, 27.87%. Found: C, 47.52%; H, 5.26%; N, 27.69%.

B. Morpholine (5.0 g., 0.06 mole) was added to a suspension of 5.1 g. (.03 mole) of 6-mercaptopurine, monohydrate in 50 ml. of anhydrous ethyl alcohol. This suspension was stirred for fifteen minutes and 2.5 g. of 37% formalin was added. This reaction mixture was then stirred overnight. The solid material was then separated by suction filtration and air dried. The yield was 6.5 g. (87%). of a material with m.p. 230° dec. The melting point of a mixture of this material and the material from (XI) A. showed no depression. The infrared spectra showed the material to be identical to (XI) A.

9-PIPERIDINOMETHYL-6-PURINETHIOL (XII). To a suspension

of 5.1 g. (.03 mole) of 6-mercaptopurine, monohydrate in 50 ml. of anhydrous ethyl alcohol was added 5.0 g. (.06 mole) of piperidine. This suspension was stirred for fifteen minutes and 2.5 g. of 37% formalin was added. This reaction mixture was stirred overnight. The solid material was then separated by suction filtration and air dried. The yield was 6 g. (80%) of crystalline material, m.p. 206-207°. Recrystallization from ethyl alcohol gave crystalline material with a m.p. 207-208°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 327 mμ (ϵ_M = 1.80 x 10^4).

Analytical calculated for $C_{11}H_{15}N_5S$: C, 52.98%; H, 6.06%; N, 28.09%. Found: C, 52.91%; H, 6.13%; N, 27.96%.

6-(n-PROPYLTHIO)-9-MORPHOLINOMETHYLPURINE (XIII)

A. To a solution of 3.92 g. (.02 mole) of 6-(n-propylthio)-purine in 60 ml. of anhydrous methanol was added 1.64 g. (.02 mole) of morpholine. This solution was stirred for fifteen minutes and then 1.62 g. of 37% formalin was added. This reaction mixture was then stirred overnight and concentrated in vacco to a thick oil. Crystallization was effected from an ethyl acetate-petroleum ether (b.p. 30-60°) mixture. The yield was 4.3 g. (73%). Recrystallization from ethyl ether gave a crystalline material with m.p. 74-75°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 290 mμ (ϵ_M = 1.80 x 10^4).

Analytical calculated for $C_{13}H_{19}N_5OS$: C, 53.22%; H, 6.53%; N, 23.87%. Found: C, 53.40%; H, 6.62%; N, 23.64%.

B. A solution of 2.51 g. (.01 mole) of 9-morpholino-

methyl-6-purinethiol (XI) in 35 ml. of 1.5 N sodium hydroxide was heated to 45° on a steam bath. To this solution was added, dropwise, a solution consisting of 1.23 g. (.01 mole) of n-bromopropane in 5 ml. of anhydrous ethyl alcohol. The resulting reaction mixture was maintained at 45-50° for 1.5 hours and then allowed to stand overnight unheated. The solution was then extracted with three 35 ml. portions of ethyl ether. The combined extracts were dried over anhydrous calcium chloride and evaporated to a thick colorless oil. The material was crystallized from ethyl ether. The yield was 2 g. (68%). Recrystallization from ethyl ether gave a crystalline material with m.p. 74-75°. The melting point of a mixture of this material and the material from XIII. A. showed no depression. The infrared spectrum of the material was identical to that of the material from XIII. A.

9-[6-(n-PROPYLTHIO-)PURINYL-]METHYL-N-PHENYL CARBAMATE (XIV). To a suspension of 2.26 g. (.01 mole) of 6-(n-propylthio)-9-hydroxymethylpurine (III) in 50 ml. of anhydrous benzene was added 1.05 g. (.01 mole) of phenyl isocyanate. The mixture was refluxed overnight. The resulting solution was cooled to 10° and the solid separated by suction filtration. The yield was 3 g. (70%). Recrystallization from benzene gave a crystalline material with m.p. 163-164°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 291 mμ (ϵ_M = 4.69 x 10^4), λ_{max} = 284 mμ (ϵ_M = 4.69 x 10^4), λ_{max} = 229 mμ (ϵ_M = 5.75 x 10^4).

Analytical calculated for $C_{16}H_{17}N_5O_2S$: C, 55.96%;

H, 4.99%; N, 20.40%. Found: C, 56.15%; H, 4.93%; N, 20.12%.

9-[6-(n-PROPYLTHIO-)PURINYL-]METHYL-N-(n-BUTYL)-CARBAMATE (XV). 6-(n-Propylthio)-9-hydroxymethyl purine (III) (4.52 g., 0.02 mole) was suspended in 15 ml. of n-butyl isocyanate. Two drops of pyridine were added and the mixture was stirred until a solution had been effected. Stirring was continued for two hours. To this solution was added 15 ml. of petroleum ether (b.p. 30-60°) and the solution was then cooled in an ice bath to effect crystallization. The crystals were collected by suction filtration. The yield was 5.8 g. (89%). Recrystallization was from an ethyl ether-petroleum ether (b.p. 30-60°) mixture. The recrystallized material had m.p. 88-89°. The ultraviolet absorption spectrum in 95% ethyl alcohol gave λ_{max} = 291 mμ ($\epsilon_M = 1.92 \times 10^4$), λ_{max} = 284 mμ ($\epsilon_M = 1.90 \times 10^4$), λ_{max} = 227 mu ($\epsilon_M = 9.5 \times 10^3$).

Analytical calculated for $C_{14}H_{21}N_5O_2S$: C, 51.99%; H, 6.55%; N, 21.66%. Found: C, 51.94%; H, 6.70%; N, 21.53%.

XVI. A. 6-Mercaptopurine, monohydrate (5.46 g., 0.032 mole) was suspended in a solution of 1.86 g. (.015 mole) of sodium carbonate, monohydrate in 35 ml. of water. To this suspension was added 2.25 ml. of 37% formalin. The mixture was stirred overnight. The solid was collected by suction filtration and air dried. The yield was 5.36 g. Recrystallization from ethyl alcohol gave a crystalline material with m.p. 320° dec. The infrared spectrum of this material was identical with that of 6-mercaptopurine, monohydrate. The melting point of a

mixture of this material and 6-mercaptopurine, monohydrate showed no depression.

B. 6-Mercaptopurine, monohydrate (3.6 g., 0.021 mole) was dissolved in 10 ml. of 2 N NaOH. To this solution was added 1.5 ml. of 37% formalin. The solution was allowed to stand for three hours and the pH adjusted to 7 with acetic acid. The solid which separated was collected by suction filtration. The yield was 3 g. This material was shown to be 6-mercaptopurine, monohydrate by melting point and infrared spectrum.

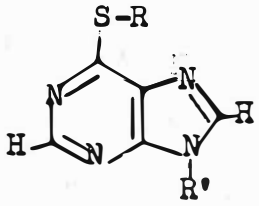
XVII. 9-Cyclopentyl-6-purinethiol (4.4 g., 0.02 mole) was suspended in 50 ml. of anhydrous ethyl alcohol. To this suspension was added, with stirring, 3.24 g. (.04 mole) of morpholine and stirring was continued for 15 minutes. To the above suspension 1.5 ml. of 37% formalin was added and stirring was continued for 10 hours. The solid material was separated by suction filtration and air dried. The yield was 4.2 g. The infrared spectrum of the material was identical with that of 9-cyclopentyl-6-purinethiol.

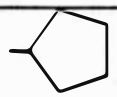
XVIII. To a solution of 2.8 g. (.02 mole) of 5, 5-dimethyl-1,3-cyclohexanedione (dimedon) in 50 ml. of ethyl alcohol was added 2.26 g. (.01 mole) of 6-(n-propylthio)-9-hydroxymethylpurine (III). The solution was stirred overnight and the solid which separated was collected by suction filtration. This material (XVIII. A.) had m.p. 189-190° (lit.[9] m.p. for dimedon-formaldehyde adduct 189-190°). The yield of XVIII. A. was 2.7 g. (93%). The supernatant liquid from the above filtration

was concentrated *in vacuo* to give an amorphous solid. This solid was dissolved in ether and the solution cooled to effect crystallization. A small amount of crystalline material separated. The liquid was decanted off and evaporated to give a white amorphous solid. This solid was washed with water and dried (XVIII. B.). This material had a m.p. of 174-176°. An authentic sample of 6-(n-propylthio)-purine had m.p. 177°. The melting point of a mixture of material XVIII. B. with 6-(n-propylthio)-purine showed no depression. The yield was 1.5 g. (77%).

TABLE I

MICRO-ANALYTICAL DATA



	R	R'	Calc'd	Found
I	$-CH_3$	$-CH_2OH$	C, 42.84 H, 4.11 N, 28.55	C, 42.85 H, 4.04 N, 28.22
II	$-CH_2CH_3$	$-CH_2OH$	C, 45.70 H, 4.79 N, 26.65	C, 45.81 H, 4.92 N, 26.49
III	$-CH_2CH_2CH_3$	$-CH_2OH$	C, 48.20 H, 5.39 N, 24.98	C, 48.41 H, 5.43 N, 25.11
IV	$-CH_2(CH_2)_2CH_3$	$-CH_2OH$	C, 50.40 H, 5.92 N, 23.51	C, 50.44 H, 5.78 N, 23.47
V	$-CH_2(CH_2)_3CH_3$	$-CH_2OH$	C, 52.35 H, 6.39 N, 22.21	C, 52.51 H, 6.55 N, 22.04
VI		$-CH_2OH$	C, 52.78 H, 5.64 N, 22.38	C, 52.98 H, 5.84 N, 22.12
VII	$-CH_2(CH_2)_4CH_3$	$-CH_2OH$	C, 54.11 H, 6.81 N, 21.04	C, 54.32 H, 7.03 N, 20.81
VIII	$-CH_2(CH_2)_5CH_3$	$-CH_2OH$	C, 55.68 H, 7.19 N, 19.98	C, 55.87 H, 7.27 N, 20.27
IX	$-CH_2(CH_2)_6CH_3$	$-CH_2OH$	C, 57.11 H, 7.53 N, 19.03	C, 57.32 H, 7.70 N, 19.01
X	$-CH_2(CH_2)_8CH_3$	$-CH_2OH$	C, 59.59 H, 8.13 N, 17.37	C, 59.60 H, 8.18 N, 17.41

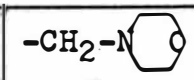
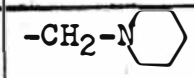
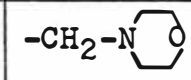
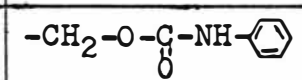
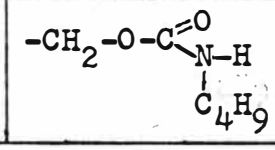
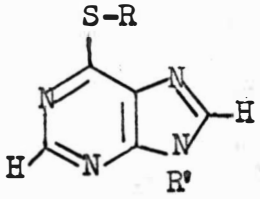
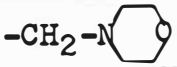
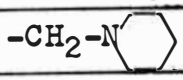
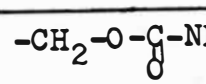
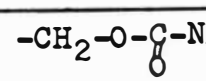
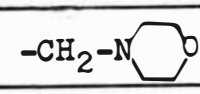
	R	R'	Calc'd	Found
XI	-H		C, 47.79 H, 5.21 N, 27.87	C, 47.52 H, 5.26 N, 27.69
XII	-H		C, 52.98 H, 6.06 N, 28.09	C, 52.91 H, 6.13 N, 27.96
XIII	$-CH_2CH_2CH_3$		C, 53.22 H, 6.53 N, 23.87	C, 53.40 H, 6.62 N, 23.64
XIV	$-CH_2CH_2CH_3$		C, 55.96 H, 4.99 N, 20.40	C, 56.15 H, 4.93 N, 20.12
XV	$-CH_2CH_2CH_3$		C, 51.99 H, 6.55 N, 21.66	C, 51.94 H, 6.70 N, 21.53

TABLE II

ULTRAVIOLET SPECTRAL DATA



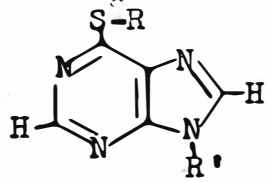
R	R'	λ_{max}*	$\epsilon_M \times 10^{-4}$
-H	-H	328	1.68
-H		325 260	2.4 0.84
-H		328	2.14
-H		327	1.80
$-CH_3$	-H	288	2.20
$-CH_3$	$-CH_2OH$	288	1.87
$-CH_2CH_3$	-H	290	2.04
$-CH_2CH_3$	$-CH_2OH$	289	1.72
$-CH_2CH_2CH_3$	-H	290	1.82
$-CH_2CH_2CH_3$	$-CH_2OH$	291	1.80
$-CH_2CH_2CH_3$		291 284 227	1.92 1.90 0.95
$-CH_2CH_2CH_3$		291 284 229	4.69 4.69 5.75
$-CH_2CH_2CH_3$		290	1.80
$-CH_2(CH_2)_2CH_3$	-H	291	1.82
$-CH_2(CH_2)_2CH_3$	$-CH_2OH$	291	1.92
$-CH_2(CH_2)_3CH_3$	-H	291	1.81

R	R'	λ max*	$\epsilon_M \times 10^{-4}$
$-CH_2(CH_2)_3CH_3$	$-CH_2OH$	292	1.93
	-H	292	1.79
	$-CH_2OH$	292	1.77
$-CH_2(CH_2)_4CH_3$	-H	291	1.81
$-CH_2(CH_2)_4CH_3$	$-CH_2OH$	292	1.99
$-CH_2(CH_2)_5CH_3$	-H	291	1.81
$-CH_2(CH_2)_5CH_3$	$-CH_2OH$	292	2.08
$-CH_2(CH_2)_6CH_3$	-H	292	1.79
$-CH_2(CH_2)_6CH_3$	$-CH_2OH$	291	2.22
$-CH_2(CH_2)_8CH_3$	-H	291	1.83
$-CH_2(CH_2)_8CH_3$	$-CH_2OH$	292	1.92

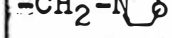
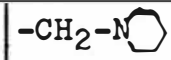
*All spectra were taken in 95% ethyl alcohol and are expressed in mμ.

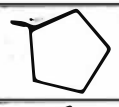
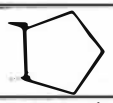
TABLE III

INFRARED SPECTRAL DATA*



All bands listed in microns

R	R'	O-H	C-O	N-H	S-H	C=S	C-S
H	H	2.89		3.25	Did not show	8.9	14.80 15.42
H			8.7		Did not show	9.0	15.25 15.60
H					Did not show	9.05	14.70 15.40
H					Did not show	9.05	15.30
$-CH_3$	-H			3.25			14.75 15.62 15.90
$-CH_3$	$-CH_2OH$	3.20 9.3	7.90				14.75 15.62 15.90
$-CH_2CH_3$	-H			3.25			14.75 15.62 15.90
$-CH_2CH_3$	$-CH_2OH$	3.17 9.3	7.40				14.75 15.50 15.80

R	R'	O-H	C-O	N-H	S-H	C=S	C-S
$-CH_2CH_2CH_3$	-H			3.25			14.75 15.50 15.90
$-CH_2CH_2CH_3$	$-CH_2OH$	3.18 9.3	7.80				14.50 15.25 15.62
$-CH_2(CH_2)_2CH_3$	-H			3.25			14.80 15.50 15.90
$-CH_2(CH_2)_2CH_3$	$-CH_2OH$	3.18 9.3	7.90				14.75 15.55 15.85
$-CH_2(CH_2)_3CH_3$	-H			3.25			14.90 15.57 15.80
$-CH_2(CH_2)_3CH_3$	$-CH_2OH$	3.17 9.20	7.90				14.80 15.57 15.85
	-H			3.28			14.75 15.60 15.90
	$-CH_2OH$	3.15 9.30	7.73				14.60 15.33 15.70
$-CH_2(CH_2)_4CH_3$	-H			3.25			14.90 15.60 15.80
$-CH_2(CH_2)_4CH_3$	$-CH_2OH$	3.17 9.20	7.98				14.75 15.55 15.82
$-CH_2(CH_2)_5CH_3$	-H			3.25			14.90 15.55 15.80
$-CH_2(CH_2)_5CH_3$	$-CH_2OH$	3.20 9.20	7.98				14.70 15.55 15.82
$-CH_2(CH_2)_6CH_3$	- H			3.25			14.90 15.55 15.80

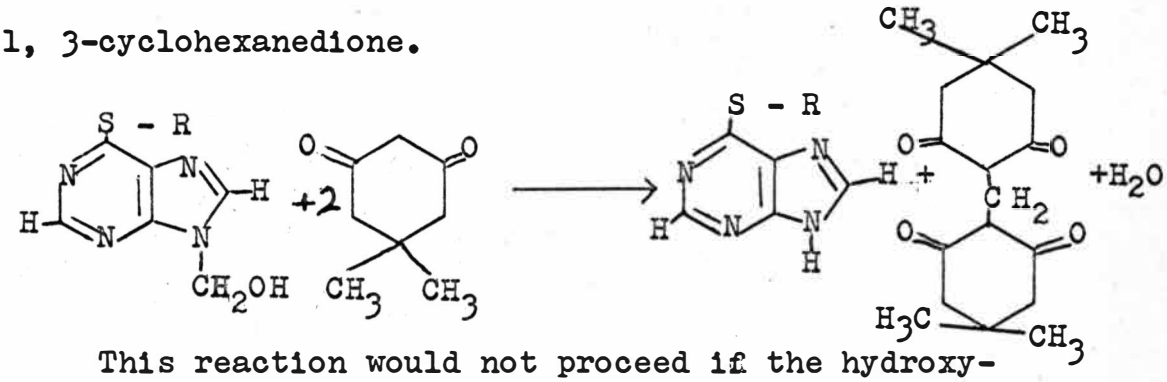
R	R'	O-H	C-O	N-H	S-H	C=S	C-S
$-CH_2(CH_2)_6CH_3$	$-CH_2OH$	3.17 9.20	7.98				14.70 15.55 15.82
$-CH_2(CH_2)_8CH_3$	-H			3.25			14.90 15.57 15.80
$-CH_2(CH_2)_8CH_3$	$-CH_2OH$	3.18 9.20	7.98				14.70 15.57 15.82

* Bands cited are those listed by Bellamy[10] and are of the same relative intensity as those listed below.

Group	λ_{max}
O-H	2.94-3.13 (v.s.) near 9.1 (s.)
C-O (alcoholic) etheral (cyclic)	7.40-7.90 (s.) 8.77-9.35 (v.s.)
N-H	2.94-3.23 (m.)
S-H	3.85-3.92 (w.)
C=S	8.33-9.52 (s.)
C-S	14.30-16.67 (w.)

DISCUSSION

Hydroxymethylation of 6-alkylthiopurines proceeds smoothly in 37% formalin solution. The products are 6-alkylthio-9-hydroxymethylpurines. The assignment of the hydroxymethyl group to the 9 position is based on the infrared and ultraviolet spectral data and the reaction of these compounds with dimedon, 5, 5-dimethyl-1, 3-cyclohexanedione.



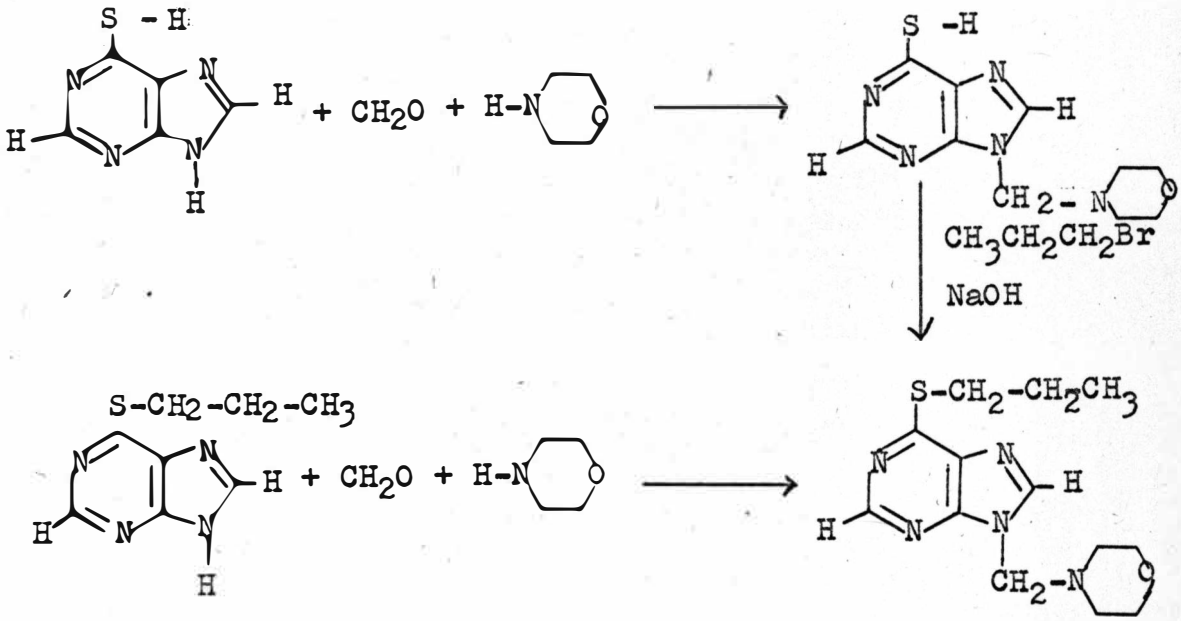
This reaction would not proceed if the hydroxymethyl group were attached to a carbon atom.[5] The fact that this reaction did proceed quantitatively eliminates the possibility of the hydroxymethylation occurring at position 8.

The ultraviolet spectral evidence supports the assigning of the 9 substitution. If the substitution was in the 7 position one would expect a large bathochromic shift; whereas, if the substitution is in the 9 position one would expect a small hypsochromic shift or no shift at all.[11] The largest shift which was observed in the ultraviolet spectra was one mμ.

The infrared spectra show the disappearance of the N-H band when the 6-alkylthiopurines are reacted with

formaldehyde alone or when reacted with formaldehyde and a secondary amine.

6-Mercaptopurine reacted with formaldehyde and secondary amines to give 9-dialkylaminomethyl-6-purinethiol. Substitution was not expected to occur in the 9 position with 6-mercaptopurine because thiophenol reacts at the mercapto group.[12] The ultraviolet spectra of 9-morpholino-methyl-6-purinethiol and 9-piperidino-methyl-6-purinethiol, however, showed a small hypsochromic shift, indicating that substitution had occurred at the 9 position. The following reaction scheme is also indicative that the substitution was



not in the 6 position. Failure of the reaction between 9-cyclopentylthiopurine, formaldehyde and morpholine is more evidence that the reaction is not occurring at the 6 or 8 position.

Butanol-water in an ammonia atmosphere for paper chromatography will show the difference between 7 and

9 substituted purines.[11] Since only one spot was obtained using this system as well as thin layer chromatography, the products are thought to be only one isomer.

The hydroxyl group of the 9-hydroxymethyl-6-alkyl-thiopurines reacts similarly to the hydroxyl group of other alcohols when treated with isocyanates. The 6-(n-propylthio)-9-hydroxymethylpurine was reacted with alkyl and aryl isocyanates to obtain the desired carbamates.

SUMMARY

In all, fifteen new compounds have been prepared. Samples of them have been sent to the Cancer Chemotherapy National Service Center for testing and evaluation in their screening program. One of the compounds, 9-piperidinomethyl-6-purinethiol (XII), has passed all the sequential stages and is now awaiting evaluation in clinical testing. The other compounds are just starting the sequential stages of testing.

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VITA

The author was born in Sampson County, North Carolina, on May 25, 1940. He received his high school education in Jackson, Ohio and Montague, Michigan. He received his A. B. degree from Western Michigan University in June, 1963.

He was married on August 22, 1964 to the former Ronna K. Rhodes.