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PUROMYCIN. SYNTHETIC STUDIES. II. THE POSITION OF GLYCOSIDATION ON THE 6-DIMETHYLAMINOPURINE MOIETY

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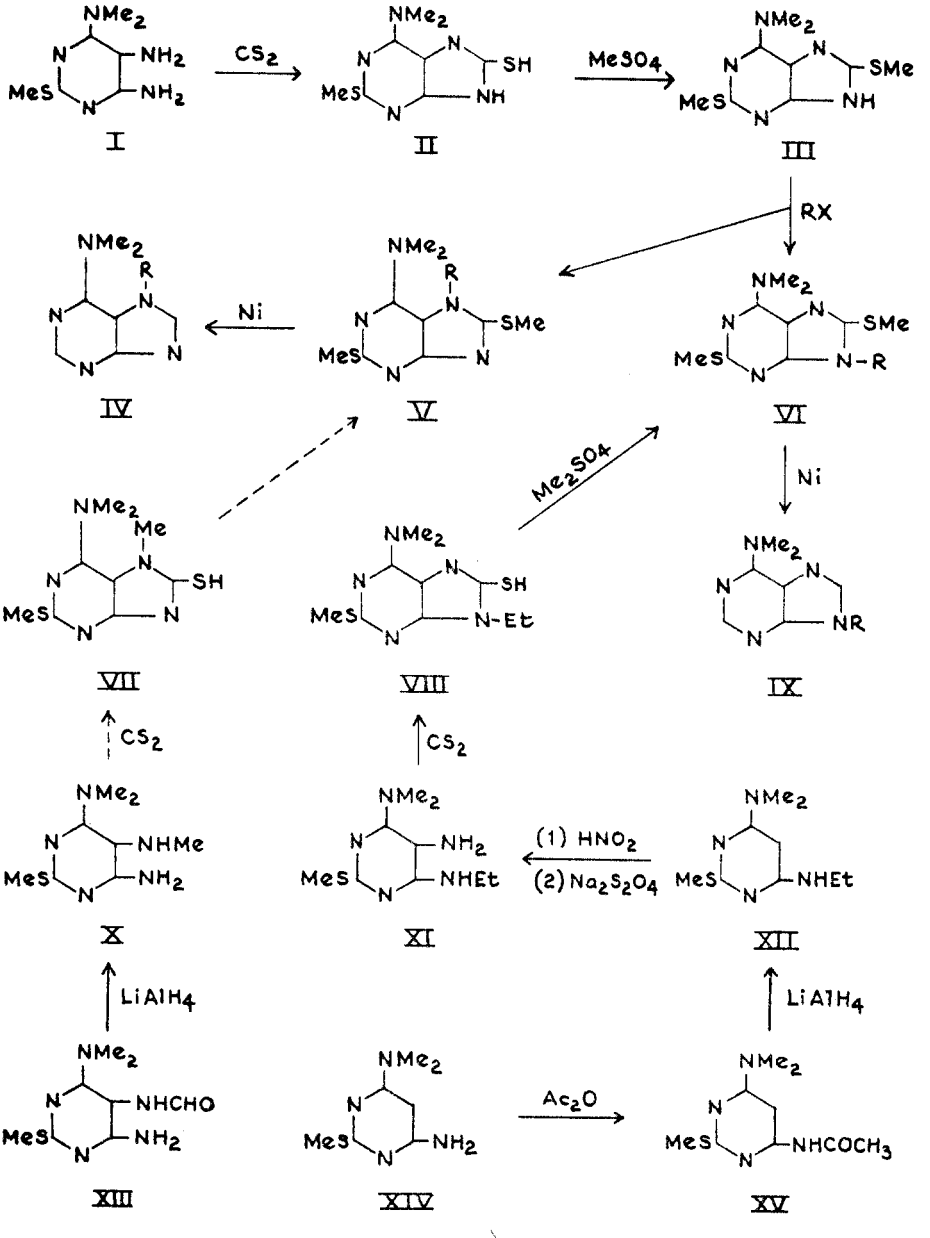
The synthesis of 6-dimethylaminopurine and its identity (1) with the $C_7H_9N_5$ moiety obtained on alcoholysis of puromycin (2) has established the purine ring system in the antibiotic. Since the remainder of the molecule is attached to the purine by a glycosidic linkage, the point of linkage must be at the 7- or 9- position of the purine nucleus. The 7- and 9- ethyl derivatives of 6-dimethylaminopurine have been synthesized unequivocally. Comparative ultraviolet absorption data has led to a definite assignment of the glycosidic linkage to the 9-position.

Reaction of 2-methylmercapto-4-amino-6-dimethylaminopyrimidine (XIV) with acetic anhydride in pyridine at 100° gave a 90% yield of amide (XV). Reduction with ethereal lithium aluminum hydride by the addition of a pyridine solution of XV resulted in a 75% yield of 2-methylmercapto-4-ethylamino-6-dimethylaminopyrimidine (XII). Nitrosation of XII on the 5-position proceeded in 96% yield. The crystalline nitroso derivative was rapidly reduced in acetone by addition of aqueous sodium hydrosulfite to the non-crystalline triamine which was immediately reacted with carbon disulfide (3) in pyridine to give the crystalline 8-mercapto-9-ethylpurine (VIII) in 74% yield from the nitroso-pyrimidine. Treatment of VIII with methyl sulfate afforded authentic 2,8-bis-methylmercapto-6-dimethylamino-9-ethylpurine (VIb), m.p. 131–132°.[1]

Ethylation of the sodium salt of 2,8-bis-methylmercapto-6-dimethylamino-purine (III) (1) with ethyl iodide gave a readily separable mixture of 35% of the 9-ethyl derivative (VIb), m.p. 131–132°, and 18% of the 7-ethyl derivative, (Vb), m.p. 161–163°. Similarly, methylation of III with methyl sulfate formed 51% of 2,8-bis-methylmercapto-6-dimethylamino-9-methylpurine (VIa), m.p. 127–128°, and 27% of the 7-methyl derivative (Va), m.p. 165–166°. The structures of the methyl derivatives are clearly indicated by comparison of their respective u.v. spectra with the authentic ethyl derivatives, Vb and VIb, as shown in Table I.

Desulfurization of VIb resulted in 74% of 6-dimethylamino-9-ethylpurine (IXb), m.p. 79–80°. Similarly, the corresponding 9-methyl derivative (IXa) was obtained in 72% yield. 6-Dimethylamino-7-ethylpurine (IVb) was isolated as the picrate after desulfurization of Vb. The corresponding 7-methylpurine (IVa) was obtained in 50% yield, m.p. 168–169°. The close similarity of the u.v. spectra of the 9-alkyl derivatives of 6-dimethylaminopurine and puromycin in contrast to the large differences between the latter and the 7-alkyl derivatives clearly prove that puromycin is a 9-substituted derivative of 6-dimethylamino-

[1] The corresponding authentic 2,8-bis-methylmercapto-6-dimethylamino-9-methylpurine could not be prepared by this approach since XIV failed to formylate.

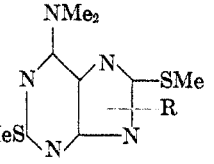


a SERIES, R=Me
b SERIES, R=Et

CHART I

TABLE I

U.V. SPECTRA[a] OF

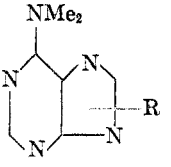


COMPOUND	MAXIMUM ($\epsilon \times 10^{-4}$) IN 5% ALCOHOL IN WATER		
	*p*H1	*p*H7	*p*H14
9-Ethyl	260 (1.22)	247.5 (2.42)	247.5 (2.27)
	305 (2.08)	302.5 (2.24)	302.5 (2.14)
9-Methyl	260 (1.33)	247.5 (2.40)	247.5 (2.40)
	305 (2.07)	302.5 (2.17)	302.5 (2.17)
7-Ethyl	260 (2.42)	252.5 (2.79)	250 (2.56)
	312.5 (2.89)	315 (2.39)	316 (2.22)
7-Methyl	260 (2.22)	252.5 (2.27)	252.5 (2.43)
	312.5 (2.75)	315 (2.17)	317.5 (2.27)

[a] All spectra determined with a Beckman model DU u.v. spectrophotometer.

TABLE II

U.V. SPECTRA OF



COMPOUND	MAXIMUM ($\epsilon \times 10^{-4}$) IN WATER		
	*p*H1	*p*H7	*p*H14
Puromycin[a]	267.5 (1.95)	275 (2.03)	275 (2.03)
9-Methyl	270 (1.75)	276 (1.81)	277 (1.81)
9-Ethyl	270 (1.75)	277.5 (1.80)	277.5 (1.83)
7-Methyl	290 (1.98)	295 (1.74)	295 (1.56)
7-Ethyl	290 (2.06)	295 (1.70)	295 (1.59)
7-Benzyl[b]	292 (2.17)		300 (1.65)

[a] From reference (2). [b] Determined on picrate in 5% alcohol. The peaks due to picric acid are omitted.

purine (Table II).[2] The molecular extinction coefficients of puromycin were 1000–2000 higher than the simple alkyl derivatives. This is due to the absorption of the phenyl ring of the O-methyl-L-tyrosyl moiety of puromycin since 6-dimethylamino-7-benzylpurine, obtained by benzylation of 6-dimethylaminopurine, has extinction coefficients 1000–2000 higher than the corresponding 7-methyl or 7-ethyl derivatives (IV).

[2] The attachment of the ribofuranosyl side-chain to the 9-position of adenine in the structure of adenosine and other nucleosides was demonstrated by Gulland and Holiday (8) by comparison of the u.v. spectra of the nucleosides with suitable 7- and 9-methylpurines.

Prior to the successful synthesis of the authentic 9-ethylpurine (VIb) from XIV as described above, an unequivocal synthesis of 2,8-bis-methylmercapto-6-dimethylamino-7-methylpurine (Va) was investigated. 2-Methylmercapto-4-amino-5-formamido-6-dimethylaminopyrimidine (XIII), obtained by formylation of the triamine (I) (1), was reduced to the 5-methylaminopyrimidine (X) by the addition of a pyridine solution of XIII to an ethereal solution of lithium aluminum hydride.[3] The common Soxhlet extraction procedure for the reduction of ether-insoluble compounds (4) was unsuccessful. Unfortunately, compound X failed to react with carbon disulfide in pyridine to give Va. It also failed to react with hot 90% formic acid to form the 5-formyl derivative which should then cyclize to a purine on heating.

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EXPERIMENTAL

2-Methylmercapto-4-acetamino-6-dimethylaminopyrimidine (XV). A solution of 5.0 g. of XIV in 15 cc. of acetic anhydride and 15 cc. of pyridine was heated on the steam-bath for one hour during which time some product separated. The mixture was cooled in an ice-bath. The solid was collected and washed with alcohol; yield, 5.5 g. (90%), m.p. 218–220°. Recrystallization of a similar preparation from alcohol gave white crystals, m.p. 219–220°.

Anal. Calc'd for $C_9H_{14}N_4OS$: C, 47.8; H, 6.24; N, 24.8.

Found: C, 47.6; H, 6.19; N, 24.9.

Attempts to formylate the amine group by heating with 98% formic acid or 1:1:1 98% formic acid-acetic anhydride-pyridine gave only unchanged amine.

2-Methylmercapto-4-ethylamino-6-dimethylaminopyrimidine (XII). (*A*). A solution of 3 g. of lithium aluminum hydride in 150 cc. of reagent ether was refluxed under a Soxhlet extractor containing 5.5 g. of finely pulverized XV in the thimble (4). After seven hours only 1.2 g. of XV had extracted. The excess hydride was decomposed by the dropwise addition of 15 cc. of ethyl acetate. Then 15 cc. of water was added dropwise followed by 58 cc. of 10% sodium hydroxide. The organic layer was decanted from the sludge and the latter was washed with two 50-cc. portions of ethyl acetate by decantation. The combined organic extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo*, leaving 0.80 g. (15% or 71% based on amide reacted) of white solid, m.p. 124–126°. Recrystallization from heptane gave white crystals, m.p. 126–127°.

Anal. Calc'd for $C_9H_{16}N_4S$: C, 51.0; H, 7.60; N, 26.4.

Found: C, 51.0; H, 7.68; N, 26.1.

(*B*). Above the center of a refluxing solution of 3 g. of lithium aluminum hydride in 150 cc. of reagent ether was added dropwise a warm solution of 4.3 g. of XV in 75 cc. of reagent pyridine over a period of 20 minutes. A gum separated which gradually became oily over a two-hour reflux period. The mixture was then processed as in procedure *A*. The crude semi-solid product remaining after evaporation of the combined extracts was triturated with water, then recrystallized from 15 cc. of methanol; yield, 3.0 g. (75%) of white crystals, m.p. 126–127°. Although some additional product was present in the filtrate, no attempt was made to isolate it.

2-Methylmercapto-4-ethylamino-5-nitroso-6-dimethylaminopyrimidine. To a solution of 1.2 g. of XII in 35 cc. of 30% acetic acid cooled in an ice-bath was added a solution of 0.48 g. of sodium nitrite in 3.5 cc. of water. A blue-green solid began to separate. After 90 minutes

[3] The triamine (I) reacted with *p*-toluenesulfonyl chloride in pyridine, gave a 5-tosyl derivative. The latter could be smoothly methylated to the 5-tosyl derivative of X, but subsequent hydrolysis to X was unsuccessful.

at 0°, the mixture was filtered and the solid was washed with water; yield, 1.31 g. (96%), m.p. 118–119°. Recrystallization of a sample from heptane afforded blue crystals, m.p. 119–120°.

Anal. Calc'd for $C_9H_{15}N_5OS$: C, 44.8; H, 6.26; N, 29.0.
Found: C, 45.0; H, 6.42; N, 29.3.

2-Methylmercapto-6-dimethylamino-8-mercapto-9-ethylpurine (VIII). To a warm solution of 500 mg. of the preceding nitroso compound in 5 cc. of acetone was added a solution of 870 mg. of sodium hydrosulfite in 5 cc. of water. The color rapidly bleached and inorganic material separated. After two minutes the mixture was diluted with 25 cc. of water. An oil separated from the solution and the mixture was extracted with three 15-cc. portions of chloroform. The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving the triamine (XI) as a dark gum which did not crystallize.

The crude triamine (XI), dissolved in 5 cc. of pyridine, was treated with 1 cc. of carbon disulfide. The solution was refluxed for 30 minutes. At the b.p. the solution became much lighter and hydrogen sulfide evolution was complete in 10 minutes. Evaporation to dryness *in vacuo* and trituration of the residue with 10% alcohol gave 410 mg. (74%) of product, m.p. 228–230°. Recrystallization of a sample from alcohol afforded white crystals, m.p. 231–233°.

Anal. Calc'd for $C_{10}H_{15}N_5S_2$: C, 44.6; H, 5.62; N, 26.0.
Found: C, 45.1; H, 5.73; N, 25.7.

2-Methylmercapto-4-amino-5-methylamino-6-dimethylaminopyrimidine (X). Above the center of a refluxing solution of 1.00 g. of lithium aluminum hydride in 100 cc. of reagent ether was added dropwise a warm solution of 1.25 g. of XIII (1) in 15 cc. of dry pyridine over a period of ten minutes. A gum separated which gradually became oily over a two-hour reflux period. The mixture was processed as described for XII. The crude residue remaining after evaporation of the organic extracts was recrystallized from alcohol; yield, 0.92 g. (79%), m.p. 163–165°. Recrystallization from the same solvent gave white crystals, m.p. 166–167°.

Anal. Calc'd for $C_8H_{15}N_5S$: C, 45.0; H, 7.10; N, 32.8.
Found: C, 45.3; H, 7.38; N, 33.0.

When this reduction was attempted by the usual Soxhlet extraction procedure for ether. insoluble compounds (4), no appreciable amount of amide dissolved after 7 hours of reflux. ing. Although pyridine supposedly reacts with the reagent (4), no difficulty was experienced.

2-Methylmercapto-4-amino-5-tosylamido-6-dimethylaminopyrimidine. To a solution of 1.00 g. of I in 3 cc. of reagent pyridine and 0.7 cc. of triethylamine was added 1.15 g. of *p*-toluenesulfonyl chloride. The mixture was heated on the steam-bath for 30 minutes, then diluted with 25 cc. of 3% sodium hydroxide. The turbid solution was clarified with Norit by filtration through Celite. The filtrate was poured into excess dilute acetic acid. The product was collected and washed with water; yield, 1.35 g. (76%), m.p. 194–198°.

If the triethylamine was omitted, 25% of the hydrochloride of I, m.p. 266° dec., separated from the pyridine solution, and was identified by conversion to the free base. The hydrochloride was analyzed.

Anal. Calc'd for $C_7H_{13}N_5S \cdot HCl$: N, 29.7. Found: N, 29.3.

When the filtrate was worked as above a 66% yield of the sulfonamide was obtained, m.p. 200–201°. Recrystallization from alcohol gave nearly white crystals, m.p. 208–209°.

Anal. Calc'd for $C_{14}H_{19}N_5O_2S_2$: C, 47.6; H, 5.42; N, 19.8.
Found: C, 47.9; H, 5.89; N, 19.6.

2-Methylmercapto-4-amino-5-N-methyltosylamido-6-dimethylaminopyrimidine. (*A*). To a solution of 1.00 g. of the above sulfonamide in 5 cc. of alcohol and 1.13 cc. of 10% aqueous sodium hydroxide was added 0.36 cc. of methyl iodide. After being refluxed for 30 minutes, the solution was neutral. The mixture was made basic with excess 10% aqueous sodium hydroxide. The product was collected and washed with water; yield, 0.91 g. (87%), m.p. 230–231° dec. Recrystallization of a sample from Methyl Cellosolve[4] afforded white crystals, m.p. 233° dec.

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[4] 2-Methoxyethanol.

Anal. Calc'd for $C_{15}H_{21}N_5O_2S_2$: C, 49.0; H, 5.76; N, 19.1.
Found: C, 49.2; H, 5.98; N, 19.4.

(*B*). When the equivalent quantity of methyl sulfate is employed, reaction is complete in 20 minutes without heat. The crude product was collected and suspended in 10 cc. of alcohol and 1 cc. of 10% sodium hydroxide. The mixture was heated to boiling, cooled and filtered; yield, 86%, m.p., 228° dec.

This compound did not hydrolyze to X, but was recovered unchanged, when heated at 50° in 96% sulfuric acid, a hydrolysis reaction usually useful for N-arylsulfonamides (5). Hydrochloric acid hydrolysis removed the methylmercapto group as expected (7).

2,8-bis-Methylmercapto-6-dimethylamino-9-ethylpurine (VIb). To a warm solution of 310 mg. of VIII in 1.2 cc. of 1 *N* methanolic sodium methoxide was added 0.11 cc. of methyl sulfate. There was some heat of reaction. After 20 minutes the solution was neutral and the product had separated. The crystals were collected and washed with 50% methanol; yield, 240 mg. (71%), m.p. 131–132°. Recrystallization from alcohol with the aid of Norit gave white crystals m.p. 131–132°.

Anal. Calc'd for $C_{11}H_{17}N_5S_2$: C, 46.6; H, 6.05; N, 24.7.
Found: C, 47.0; H, 5.90; N, 24.5.

2,8-bis-Methylmercapto-6-dimethylamino-7- and 9-ethylpurine (Vb and VIb). A hot solution of 4.9 g. of III (1) in 19.3 cc. of 1 *N* methanolic sodium methoxide was refluxed with 1.96 cc. of ethyl iodide for 45 minutes when the soln. had changed to *p*H 8. The solution was cooled, and the solid was collected and washed with ice-cold alcohol; wt., 2.1 g., m.p. 124–126° (turbid). This solid was dissolved in 20 cc. of hot benzene. The solution was filtered from 230 mg. (4.7%) of starting material (III). The benzene solution was evaporated to dryness *in vacuo* leaving 1.7 g. (31%) of the 9-ethyl isomer (VIb), m.p. 126–128°. A mixture with authentic VIb prepared above gave no depression in m.p.

The filtrate from 2.0 g. was diluted with water. The solid was collected; wt. 1.52 g., m.p. 132–152° (turbid). The filtrate slowly deposited 490 mg. (total 18%) of starting material. The 1.52 g. was dissolved in 20 cc. of benzene, filtered from insoluble starting material, diluted with 20 cc. of heptane, and cooled. The 7-ethyl isomer (Vb) was collected; yield, 1.0 g. (18%), m.p. 158–163°. Recrystallization from benzene-heptane gave white crystals, m.p. 161–163°.

Anal. Calc'd for $C_{11}H_{17}N_5S_2$: C, 46.6; H, 6.05; N, 24.7.
Found: C, 46.4; H, 5.99; N, 24.9.

From the filtrate of the 1.0 g. of Vb was isolated an additional 0.23 g. (4%) of VIb, m.p. 119–122°, by evaporation and trituration of the filtrate residue with ice-cold methanol.

2,8-bis-Methylmercapto-6-dimethylamino-7- and 9-methylpurine (Va and VIa). To a hot solution of 2.1 g. of III (1) in 8.3 cc. of 1 *N* methanolic sodium methoxide was added 0.77 cc. of methyl sulfate. After 15 minutes the nearly neutral mixture was filtered and the solid was washed with methanol, then water; yield, 0.985 g. (45%) of VIa, m.p. 124–125°. The combined filtrate and washings, which were now about 50% in methanol, were cooled in an ice-bath and the gum soon solidified. The solid was collected and washed with water; wt., 0.93 g. For separation, 0.88 g. of this material was dissolved in 13 cc. of hot benzene, filtered from 80 mg. of starting material, then diluted with 13 cc. of heptane. Cooling gave 0.57 g. (27%) of Va, m.p. 163–165°. The filtrate was evaporated to dryness *in vacuo*. Trituration of the residue with ice-cold alcohol gave 0.135 g. (total 51%) of VIa, m.p. 123–125°.

Recrystallization of a sample of VIa from alcohol gave white crystals, m.p. 127–128°. Recrystallization of a sample of Va from benzene-heptane gave white crystals, m.p. 165–166°. For u.v. data see Table I.

Anal. Calc'd for $C_{10}H_{15}N_5S_2$: C, 44.6; H, 5.62; N, 26.0.
Found (VIa): C, 44.5; H, 5.61; N, 26.3.
Found (Va): C, 44.9; H, 5.94; N, 26.2.

6-Dimethylamino-9-methylpurine (IXa). A solution of 700 mg. of VIa in 70 cc. of absolute alcohol was refluxed with 2 teaspoons of Raney nickel (6) for 30 minutes, then filtered hot through Celite and the catalyst was washed with absolute alcohol. The combined filtrate and washings were evaporated to dryness *in vacuo* leaving 330 mg. (72%) of white solid,

m.p. 110–113°. Recrystallization from heptane afforded white crystals, m.p. 114–115°, which were readily soluble in water.

Anal. Calc'd for $C_8H_{11}N_5$: C, 54.2; H, 6.21; N, 39.5.
Found: C, 54.4; H, 6.56; N, 39.6.

Similarly, desulfurization of 515 mg. of Va gave 170 mg. (50%) of *6-dimethylamino-7-methylpurine* (IVa), m.p. 161–163°. Recrystallization from benzene-heptane afforded white crystals, m.p. 168–169°.

Anal. Calc'd for $C_8H_{11}N_5$: C, 54.2; H, 6.21; N, 39.5.
Found: C, 54.4; H, 6.50; N, 39.4.

In the same fashion desulfurization of 1.00 g. of VIb gave 0.50 g. (74%) of *6-dimethylamino-9-ethylpurine* (IXb), m.p. 75–78°. Recrystallization from heptane afforded white crystals, m.p. 79–80°.

Anal. Calc'd for $C_9H_{13}N_5$: C, 56.5; H, 6.85; N, 36.6.
Found: C, 56.5; H, 6.84; N, 36.1.

A similar desulfurization of 2,8-bis-methylmercapto-6-amino-9-methylpurine to 9-methyladenine has been described by Cook and Smith (3).

6-Dimethylamino-7-ethylpurine (IVb). Desulfurization of 500 mg. of Vb with 1.5 teaspoons of Raney nickel gave 140 mg. (42%) of solvated white solid, m.p. 110–113° with gradual softening from 65–110°. Recrystallization from heptane gave solvated white crystals, m.p. 74–76°. The material was dried in a high vacuum for several hours and the m.p. rose to 129–130°. Further drying in a high vacuum at 80° gave a constant m.p. 135–136°. This material was still slightly solvated. The u.v. data are recorded in Table II.

Anal. Calc'd for $C_9H_{13}N_5$: C, 56.5; H, 6.85; N, 36.6.
Found: C, 56.0; H, 6.93; N, 34.8.

To obtain proper analytical values the base dissolved in water was treated with excess 1% aqueous picric acid. The *picrate* was collected and washed with water: yellow crystals, m.p. 181–183°. Recrystallization from alcohol narrowed the m.p. to 182–183°.

Anal. Calc'd for $C_9H_{13}N_5 \cdot C_6H_3N_3O_7$: C, 42.9; H, 3.84; N, 26.7.
Found: C, 43.0; H, 3.94; N, 26.6.

6-Dimethylamino-7-benzylpurine. To a solution of 210 mg. of 6-dimethylaminopurine (1) in 2.1 cc. of methanol and 1.29 cc. of 1 *N* methanolic sodium methoxide was added 0.16 cc. of benzyl chloride. The solution was refluxed for five hours when it had dropped to about *p*H 8. The mixture was diluted with water and steam-distilled *in vacuo* until the excess benzyl chloride was removed. The water insoluble gum was extracted with chloroform. Dried with magnesium sulfate, the combined extracts were evaporated *in vacuo* leaving 290 mg. (89%) of a mixture of the 7- and 9-benzylated purines as a gum. The isomers could not be separated by crystallization. This gum, dissolved in 3 cc. of alcohol, was treated with 3 cc. of saturated alcoholic picric acid. The *picrate* was collected and washed with alcohol; yield, 265 mg. (48%), m.p. 180–184° dec. Recrystallization from 50% methanol gave yellow crystals, m.p. 187–188° dec. The u.v. data in Table II clearly show that the picrate is formed from the 7-benzyl isomer.

Anal. Calc'd for $C_{14}H_{15}N_5 \cdot C_6H_3N_3O_7$: C, 49.8; H, 3.76; N, 23.2.
Found: C, 50.1; H, 4.20; N, 23.3.

SUMMARY

Some 7- and 9-alkyl derivatives of 6-dimethylaminopurine have been synthesized unequivocally. Comparison of their ultraviolet spectra with those of puromycin clearly demonstrates that in the antibiotic the 6-dimethylaminopurine moiety is glycosidated on the 9- position.

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