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PUROMYCIN. SYNTHETIC STUDIES. IV. GLUCOSYL DERIVATIVES OF 6-DIMETHYLAMINOPURINE

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Puromycin has been shown to be a derivative of 6-dimethylamino-9-(3′-aminoribosyl)purine (1). In order to effect a total synthesis of this antibiotic, it would be necessary to establish conditions for glycosidation of 6-dimethylaminopurine on the 9-position. This has now been accomplished with α-bromoacetoglucose.

The descriptions of reaction of haloaceto sugars with heavy metal salts of purines are numerous in the literature, dating back to the pioneer work of Fischer and Helferich (2). The orientation of the incoming sugar is apparently independent of the sugar, but is determined by the nature of the purine. For example, 2,8-dichloroadenine and N-acetyladenine orientate to the 9-position (2, 3), whereas the N-methylated purine, theophylline, orientates to the 7-position (4). Therefore, a study of the condensation of α-bromoacetoglucose with 6-dimethylaminopurine, its 2-methylmercapto derivative, and its 2,8-bis-methylmercapto derivative (5) was undertaken to determine their respective orientations.

Davoll and Lowy (3) have recently shown that chloromercury derivatives of purines usually give much higher yields of nucleosides than the earlier used silver or lead salts. The chloromercury derivatives of the three 6-dimethylaminopurines were prepared essentially according to their method. The most important change was the addition of Celite before filtration of the chloromercury derivative which greatly aided in filtration and drying. The Celite had a more important effect in the condensation reaction with α-bromoacetoglucose. It prevented coating over of the chloromercury derivative by gummy by-products and thus gave higher yields. For example Davoll and Lowy (3) have recorded a 22% over-all yield for the coupling of α-bromoacetoglucose with the chloromercury derivative of N-acetyladenine and subsequent deacetylation. Their procedure in this laboratory gave even lower yield, but the use of Celite raised the over-all yield to 27%.

Condensation of α-bromoacetoglucose with the chloromercury derivative of 6-dimethylaminopurine (VII) gave a 26% yield of a crystalline nucleoside acetate. That the sugar residue had entered the 7-position of the purine to give V is clearly shown by the comparison of its u.v. spectra with those of authentic 7- and 9-ethyl-6-dimethylaminopurines (6) (Table I). Thus, it became apparent that the puromycin molecule could not be reconstructed from its own purine moiety, but that a derivative of 6-dimethylaminopurine giving the desired 9-orientation would have to be found.

Condensation of the chloromercury derivative of 2,8-bis-methylmercapto-6-

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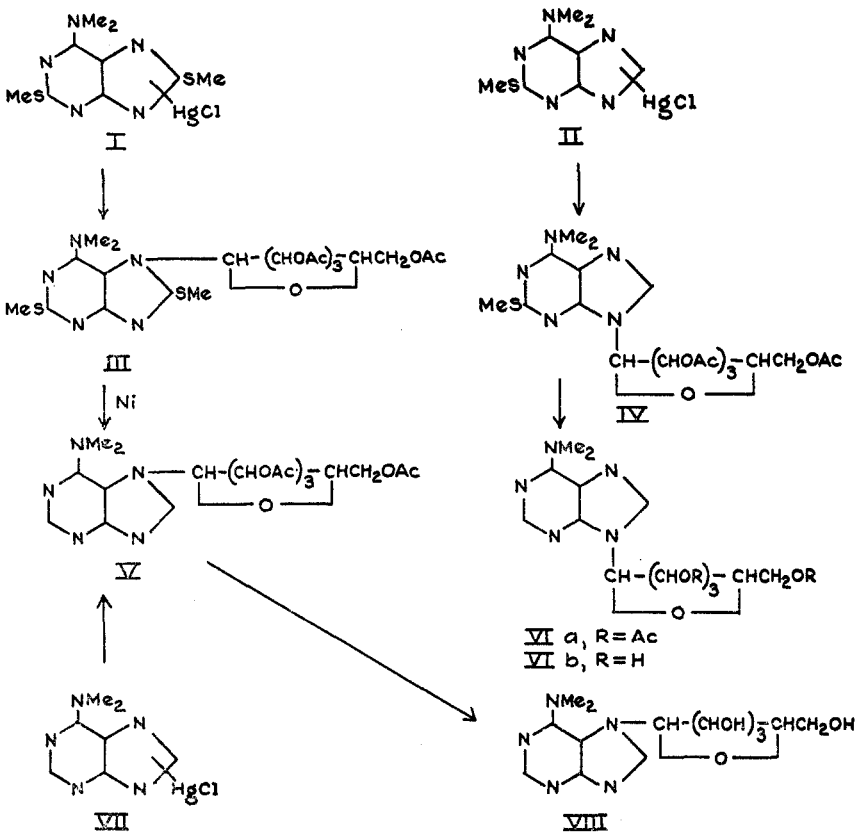
dimethylaminopurine (I) with α-bromoacetoglucose gave a crystalline nucleoside acetate in 64% yield. The u.v. spectra of this glycoside were in closer agreement with the spectra of 2,8-bis-methylmercapto-6-dimethylamino-7-ethylpurine than with those of the corresponding 9-ethyl derivative (6), indicating that the glycoside had structure III, but the agreement was not as close as for V and 6-dimethylamino-7-ethylpurine. The resonating system of III may become affected with resultant change in the normal u.v. spectra by the steric interaction of the 7-glycoside with the neighboring 6-dimethylamino and 8-methylmercapto groups. That the glycoside had structure III was further demonstrated by Raney nickel desulfurization (7) to V in 57% yield, identical with that obtained by glycosidation of the chloromercury derivative of 6-dimethylaminopurine.

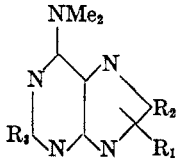


Fortunately, the chloromercury derivative of 2-methylmercapto-6-dimethylaminopurine (II) coupled with α-bromoacetoglucose to give 2-methylmercapto-6-dimethylamino-9-β-D-glucopyranosylpurine tetraacetate[2] (IV) in 49% yield.

[2] Although the reasonable assumption that α-bromoacetoglucose gives a product with β-configuration on condensation with a purine has been made for many years (2), only recently Clark, Todd, and Zussman (8) have proven unquestionably that this is true. An unequivocal chemical structure proof that adenosine has the β-configuration was offered by them. Since in that laboratory it had earlier been proven that adenosine and synthetic

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF



R_1	R_2	R_3	Maximum ($E \times 10^{-4}$) in 10% alcohol					
			pH 1		pH 7		pH 14	
7-Ethyl	MeS	MeS	260	(2.42)	252.5	(2.79)	250	(2.56)
			312.5	(2.89)	315	(2.39)	316	(2.22)
9-Ethyl	MeS	MeS	260	(1.22)	247.5	(2.42)	247.5	(2.27)
			305	(2.08)	302.5	(2.24)	302.5	(2.14)
7-β-D-Glucopyranosyl tetraacetate	MeS	MeS	267.5	(1.70)	257.5	(1.93)	255	(2.15)
			322.5	(2.39)	330	(1.76)	327.5	(1.86)
7-Ethyl	H	MeS	255	(2.08)	249	(2.67)	250	(2.47)
			275	(1.88)				
			292	(2.07)	302.5	(1.70)	300	(1.79)
9-Ethyl	H	MeS	255	(1.78)	247.5	(2.42)	247.5	(2.36)
			290	(1.45)	285	(1.60)	287.5	(1.77)
9-β-D-Glucopyranosyl tetraacetate	H	MeS	237.5	(1.38)	249	(2.50)	247.5	(2.48)
			275	(1.79)	285	(1.81)	286	(1.85)
7-Ethyl	H	H	290	(2.06)	295	(1.70)	295	(1.55)
9-Ethyl	H	H	270	(1.75)	277.5	(1.80)	277.5	(1.83)
7-β-D-Glucopyranosyl tetraacetate	H	H	292	(1.72)	301	(1.21)	298	(1.27)
7-β-D-Glucopyranosyl	H	H	292.5	(1.91)	297	(1.47)	297.5	(1.44)
9-β-D-Glucopyranosyl tetraacetate	H	H	267.5	(1.56)	273	(1.56)	275	(1.60)
9-β-D-Glucopyranosyl	H	H	268	(1.86)	275	(1.88)	275	(1.88)
Puromycin (1)			267.5	(1.95)	275	(2.03)	275	(2.03)

That the 9-glycoside had formed was again demonstrated by comparison of its u.v. spectra with those of authentic 2-methylmercapto-6-dimethylamino-7- and 9-ethylpurines.[3] Desulfurization of IV to crystalline 6-dimethylamino-9-β-D-glucopyranosylpurine tetraacetate (VIa), isomeric to V, proceeded in 44% yield. This 9-glycoside had u.v. peaks in acid and alkali identical with those of puromycin (1) and essentially the same as those of 6-dimethylamino-9-ethylpurine (Table I).

Deacetylation of the crude desulfurization product, VIa, with methanolic sodium methoxide gave 6-dimethylamino-9-β-D-glucopyranosylpurine (VIb), m.p. 249–251°, in 63% yield over-all for the two steps. Deacetylation of V gave a 76% yield of 6-dimethylamino-7-β-D-glucopyranosylpurine (VIII), m.p.

9-D-glucopyranosyladenine have the same configuration (9), it follows that the latter must also have the β-configuration.

[3] Formylation of 2-methylmercapto-4-ethylamino-5-amino-6-dimethylaminopyrimidine (6) followed by ring closure at 250° gave authentic 2-methylmercapto-6-dimethylamino-9-ethylpurine. Ethylation of the sodium salt of 2-methylmercapto-6-dimethylaminopurine gave a mixture of 7- and 9-ethyl derivatives from which the higher melting 7-isomer was readily separated.

239–241°. Thus, it should be possible to introduce any acetohalo sugar on either the 7- or 9-position of 6-dimethylaminopurine at will.

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EXPERIMENTAL

2-Methylmercapto-4-ethylamino-5-formamido-6-dimethylaminopyrimidine. The crude 2-methylmercapto-4-ethylamino-5-amino-6-dimethylaminopyrimidine obtained by the hydrosulfite reduction of 1.3 g. of 2-methylmercapto-4-ethylamino-5-nitroso-6-dimethylaminopyrimidine (6) was dissolved in 15 cc. of 90% formic acid and heated on the steam-bath for one hour. The solution was concentrated to about 3 cc. *in vacuo*, diluted with 5 cc. of water, and poured into ice and excess ammonia water. The solid was collected and washed with water; yield, 530 mg. Additional material was obtained by extraction of the filtrate with chloroform. The combined crude products were recrystallized from chloroform-heptane to give 640 mg. (47%) of product, m.p. 137–138°. Further recrystallization from the same solvents gave nearly white crystals of the same m.p.

Anal. Calc'd for $C_{10}H_{17}N_5OS$: C, 47.1; H, 6.70; N, 27.5.
Found: C, 47.2; H, 6.84; N, 27.4.

2-Methylmercapto-6-dimethylamino-9-ethylpurine. When 100 mg. of pure 2-methylmercapto-4-ethylamino-5-formamido-6-dimethylaminopyrimidine was heated in a bath at 240–250°, water evolution was complete in ten minutes. The cooled residue solidified to give 90 mg. (97%) of product, m.p. 75–77°. Several recrystallizations from heptane gave white crystals, m.p. 80–82°.

Anal. Calc'd for $C_{10}H_{15}N_5S$: C, 50.6; H, 6.34; N, 29.5.
Found: C, 50.4; H, 6.30; N, 29.7.

2-Methylmercapto-6-dimethylamino-7-ethylpurine. To a hot solution of 3.9 g. of 2-methylmercapto-6-dimethylaminopurine (5) in 18.7 cc. of 1 *N* sodium methoxide was added 1.8 cc. of ethyl iodide. The solution was refluxed on the steam-bath for 45 minutes when it was only slightly alkaline. The solution was evaporated to dryness *in vacuo* and the residue was partitioned between 20 cc. of chloroform and 10 cc. of water. Some insoluble material was removed by filtration. The chloroform solution, dried with magnesium sulfate, was evaporated to dryness *in vacuo*. The residue (3 g.) was repeatedly extracted with hot heptane. The extracts deposited about 200 mg. of crude product, m.p. 130–145°. Further recrystallization from heptane gave white crystals, m.p. 154–156°.

Anal. Calc'd for $C_{10}H_{15}N_5S$: C, 50.6; H, 6.34; N, 29.5.
Found: C, 50.6; H, 6.31; N, 29.5.

The heptane filtrates, containing both 7- and 9-ethylpurine, were not investigated further.

*2,8-bis-Methylmercapto-6-dimethylamino-7-β-*D*-glucopyranosylpurine tetraacetate* (III). To a hot solution of 3.0 g. of 2,8-bis-methylmercapto-6-dimethylaminopurine in 11.7 cc. of 1 *N* sodium methoxide was added a hot solution of 3.19 g. of mercuric chloride in 5 cc. of absolute alcohol. The chloromercury purine (I) immediately separated. The cooled mixture was filtered and the solid was washed with alcohol, water, and alcohol; yield, 4.13 g. (72%).

A mixture of 1.84 g. of I, 2.0 g. of Celite, and 100 cc. of xylene was refluxed under a constant water separator until the traces of water were removed. After the addition of 2.0 g. of α-bromoacetoglucose (10), the mixture was refluxed and stirred for one hour. The cooled solution was filtered and the precipitate (almost completely Celite) was washed with xylene. The filtrate was concentrated *in vacuo* to about 30 cc. and diluted with 200 cc. of petroleum ether. The solid (3.5 g.) was collected and dissolved in 25 cc. of hot alcohol. The solution was poured into 20 cc. of 30% potassium iodide solution. The solid was collected and washed with 50% alcohol; yield, 1.5 g. (68%), m.p. 202–206°. Recrystallization from alcohol gave white crystals, m.p. 202–204°.

Anal. Calc'd for $C_{23}H_{31}N_5O_9S_2$: C, 47.2; H, 5.83; N, 12.0.
Found: C, 46.9; H, 5.50; N, 11.8.

6-Dimethylamino-7-β-D-glucopyranosylpurine tetraacetate (V). (*A*). To a solution of 3.0 g. of 6-dimethylaminopurine dihydrochloride (1) in 25 cc. of water containing 1.5 g. of sodium hydroxide was added a solution of 3.5 g. of mercuric chloride in 15 cc. of absolute alcohol. The white chloromercury derivative (VII) was collected and washed with water, then alcohol; yield, 4.7 g. (92%).

A mixture of 1.49 g. of VII, 2.0 g. of Celite, and 125 cc. of xylene was condensed with 2.0 g. of α-bromoacetoglucose as described for III. The crude product which separated when petroleum ether was added to the concentrated xylene solution was dissolved in chloroform and the solution was washed with 30% potassium iodide solution. Dried with magnesium sulfate, the chloroform solution was evaporated to dryness *in vacuo* leaving 1.47 g. of a gum. This gum was extracted with several 5-cc. portions of hot water. On cooling, the extracts deposited 0.47 g. (26%) of product, m.p. 140–144°. Recrystallization from water gave white crystals, m.p. 147–149°, $[\alpha]_D^{24}$ −32.5° (1.9% in $CHCl_3$).

Anal. Calc'd for $C_{21}H_{27}N_5O_9 \cdot \frac{1}{2}H_2O$: C, 50.4; H, 5.66; N, 13.9.
Found: C, 50.1; H, 5.75; N, 13.9.

A sample dissolved in 50% alcohol and treated with excess 1% picric acid gave yellow crystals of a *picrate*, m.p. 193–194° dec.

(*B*). A solution of 300 mg. of III in 30 cc. of absolute alcohol was refluxed with ⅛ teaspoon of desulfurizing Raney nickel (7) for 3 hours, then filtered hot through Celite. The combined filtrate and washings were evaporated to dryness *in vacuo*. Trituration with 3 cc. of warm water, then cooling gave 144 mg. (57%) of product, m.p. and mixture m.p. with preparation *A*, 146–148°.

2-Methylmercapto-6-dimethylamino-9-β-D-glucopyranosylpurine tetraacetate (IV). The chloromercury salt, II, was prepared from its purine as described for I except that 2 g. of Celite was added for each 1 g. of purine base before filtration. The preparation of this important intermediate was simplified in the following manner:

A mixture of 18.6 g. of 2-methylmercapto-4-amino-5-formamido-6-dimethylaminopyrimidine (6), 220 cc. of alcohol, and 80 cc. of 5% sodium hydroxide was refluxed for 30 minutes. To the hot solution of the purine sodium salt was added a hot solution of 17.8 g. of mercuric chloride in 50 cc. of alcohol. The white chloromercury derivative separated. After the addition of 30 g. of Celite, the mixture was slurried, filtered, and the solid washed with water, then dried on the steam-bath; total weight, 53 g. or a yield of 23 g. (78%) of II.

A mixture of 4.17 g. of II, 6 g. of Celite, and 300 cc. of xylene was refluxed and stirred under a constant water separator until anhydrous. After the addition of 5.0 g. of α-bromoacetoglucose, the mixture was refluxed and stirred for one hour. The mixture was filtered hot and the filter cake was washed with hot chloroform until the washings were colorless. The combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was partitioned between 50 cc. of chloroform and 50 cc. of 30% potassium iodide, then filtered from some unalkylated purine base. The separated chloroform layer, dried with magnesium sulfate, was evaporated to dryness *in vacuo*. Recrystallization of the residue from 20 cc. of alcohol with the aid of Norit gave 2.5 g. (49%) of product, m.p. 169–171°. Further recrystallization of a sample from alcohol gave white crystals, m.p. 169–171°.

Anal. Calc'd for $C_{22}H_{29}N_5O_9S$: C, 49.0; H, 5.42; N, 13.0.
Found: C, 49.2; H, 5.57; N, 13.3.

6-Dimethylamino-9-β-D-glucopyranosylpurine tetraacetate (VIa). A solution of 500 mg. of IV in 40 cc. of absolute ethanol was refluxed with 3 cc. of desulfurizing Raney nickel (7) for 30 minutes, then filtered hot through Celite. The combined filtrate and washings were evaporated to dryness *in vacuo*. Crystallization from hot water by decantation from the insoluble gum followed by cooling afforded 200 mg. (44%) of white crystals, m.p. 141–143°. Further recrystallization from water did not change the m.p. This material had $[\alpha]_D^{24}$ −16.8° (1% in $CHCl_3$).

Anal. Calc'd for $C_{21}H_{27}N_5O_9$: C, 51.2; H, 5.53; N, 14.4.
Found: C, 51.2; H, 5.48; N, 14.1.

6-Dimethylamino-9-β-D-glucopyranosylpurine (VIb). Desulfurization of 2.5 g. of IV in 200 cc. of absolute alcohol with 3 teaspoons of desulfurizing Raney nickel as described above gave 1.74 g. of a crude VIa as a glass. This material was refluxed with 34 cc. of methanol and 0.34 cc. of 1 *N* methanolic sodium methoxide for 30 minutes. The solution was evaporated to dryness *in vacuo* leaving a crystalline residue, m.p. 253–254°. Recrystallization by solution in the minimum of hot water and addition of 10 volumes of absolute alcohol gave 950 mg. (63%) of product in two crops, m.p. 255–257°. When a sample was recrystallized from absolute alcohol, white crystals were formed with m.p. 249–251° and $[\alpha]_D^{26}$ −21.1° (2% in pyridine). The compound was a monohydrate which on drying in high vacuum at 140° gave the anhydrous material.

Anal. Calc'd for $C_{13}H_{19}N_5O_5$: C, 48.0; H, 5.88; N, 21.5.
Found: C, 48.2; H, 6.04; N, 21.6.

6-Dimethylamino-7-β-D-glucopyranosylpurine (VIII). A solution of 130 mg. of V in 5 cc. of methanol and 0.05 cc. of 1 *N* methanolic sodium methoxide was refluxed for 45 minutes. After about 5 minutes, the product began to separate. The mixture was cooled and filtered; yield, 53 mg. (62%), m.p. 239–240° dec. By concentration of the filtrate was obtained an additional 12 mg. of the same m.p. (total yield, 76%). Recrystallization from a large volume of methanol by concentration gave white crystals, m.p. 239–241° dec.

Anal. Calc'd for $C_{13}H_{19}N_5O_5$: C, 48.0; H, 5.88; N, 21.5.
Found: C, 48.1; H, 6.13; N, 21.5.

2-Methylmercapto-6-dimethylamino-9-β-D-glucopyranosylpurine. To a solution of 700 mg. of IV in 75 cc. of methanol and 2 cc. of Methyl Cellosolve[4] cooled to 5° was added 150 cc. of saturated methanolic ammonia. After 24 hours at 3° the solution was evaporated to dryness *in vacuo* leaving a white solid. Recrystallization from 50 cc. of water with the aid of Norit gave 0.24 g. (51%) of white crystals, m.p. 237–239°, $[\alpha]_D^{26}$ −8.0° (2% in pyridine). No attempt was made to isolate a second crop.

Anal. Calc'd for $C_{14}H_{21}N_5O_5S$: C, 45.3; H, 5.70; N, 18.9.
Found: C, 45.5; H, 5.84; N, 18.9.

A similar attempt to deacetylate III did not give a crystalline product.

SUMMARY

6-Dimethylaminopurine, the purine moiety of puromycin, and 2,8-bismethylmercapto-6-dimethylaminopurine orientate α-bromoacetoglucose to the 7-position during coupling, whereas 2-methylmercapto-6-dimethylaminopurine orientates to the 9-position. The use of these observations for the total synthesis of puromycin is discussed.

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[4] Trade name for the monomethyl ether of ethylene glycol.