[CONTRIBUTION FROM THE LEDERLE LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Puromycin. Synthetic Studies. XII.[1] Synthesis of the α-Anomer of 6-Dimethylamino-9-(3′-amino-3′-deoxy-D-ribofuranosyl)-purine

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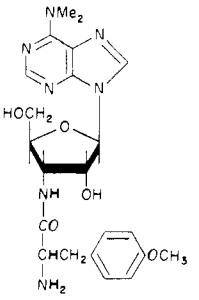
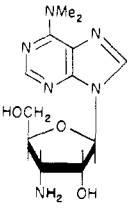
The title compound, the anomer of the biologically active "aminonucleoside" from puromycin, has been synthesized by a stereochemically controlled sequence *via* the key intermediate, 6-dimethylamino-9-(3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine (X).

The partial synthesis of the aminonucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (II), from the antibiotic, puromycin



I



II

(I) has been described in paper VII of the series[2] and the total synthesis from D-xylose in papers VIII[3a] and IX[3b] of this series. The aminonucleoside II has been shown to be highly effective against the transplanted adenocarcinoma of the C_3H mouse[4] and against *Trypanosoma equiperdum* in the mouse.[5] One of the points of the relationship of the structure of II to biological activity would be the configuration at the 1-position of the sugar. The natural isomer II has the β-configuration; the synthesis of the α-anomer (XIII) by a stereochemically controlled sequence is described in this paper.[6]

It has been postulated previously in paper V of this series[7] that the synthesis of a nucleoside from a 1-haloacyl sugar and a heavy metal salt of a purine forms nucleosides which have a C_1–C_2-*trans* configuration, regardless of the relative configuration at C_1–C_2 of the original chloro sugar. All the examples of synthetic nucleosides prepared by this method described in the literature, have so far conformed to this theory. Thus, chloroaceto-D-ribofuranose and 6-acetaminopurine mercuric chloride gave, after deacetylation, adenosine which has a C_1–C_2-*trans* configuration and is a β-anomer.[8] Similarly, 1-chloro-2,5-dibenzoyl-3-acetamino-3-deoxy-D-ribofuranose and 2-methylmercapto-6-dimethylaminopurine mercuric chloride formed, after desulfurization and debenzoylation, 6-dimethylamino-9-(3′-acetamino-3′-deoxy-β-D-ribofuranosyl)-purine, which has again a C_1–C_2-*trans* configuration and is a β-anomer.[3] In contrast, chloroaceto-D-arabinofuranose condensed with 2,6-dichloroadenine and theophylline to give nucleosides *which still had the C_1–C_2-trans configuration*, but were α-anomers.[9] It therefore follows that in order to synthesize a nucleoside such as the α-riboside XIII by this process, it would be necessary to start with a 1-chloroacylated-3-amino-3-deoxy-D-arabinose in order to obtain a nucleoside X with the α-configuration. If the 2′-hydroxyl group could then be inverted by neighboring participation of the 3′-acetamino group,[2,10] an over-all stereochemically controlled synthesis of the α-riboside XIII could be realized. This sequence did indeed prove to be successful.

Benzoylation of methyl 3-acetamino-3-deoxy-α-D-arabinofuranoside (III)[2] with benzoyl chloride in pyridine gave the benzoate IV as a glass in quantitative yield. Hydrolysis of the methyl ether with a mixture of concentrated hydrochloric acid and acetic acid at 50° afforded crystalline 2,5-dibenzoyl-3-acetamino-3-deoxy-D-arabinose (V), $[\alpha]^{22}D$ −25.6°, in 54% yield, which probably has the β-configuration. The same over-all yield of V could be obtained from the β-anomer of III.[2] Acetylation of V with acetic anhydride in pyridine at 100° gave a mixture of VIII and its β-anomer, of which one anomer, probably the α, crystallized in 75% yield and had $[\alpha]^{24}D$ +29.1°.

Reaction of VIII with titanium tetrachloride in boiling ethylene dichloride gave a titanium chloride complex[3] of 1-chloro-2,5-dibenzoyl-3-acetamino-3-deoxy-D-arabinofuranose (VII) which was condensed, without isolation, with 2-methylmercapto-6-dimethylaminopurine 9-mercuric chloride[11] forming an 86% yield of a glass, presumably VI. This was desulfurized with Raney nickel to IX in 64% yield. Debenzoylation gave a 36% yield of a mixture of two isomers, m.p. 236° and 191°. The lower melting isomer was shown to be a nucleoside of 3-amino-3-deoxy-D-arabinofuranose by benzoyla-

(1) This paper was presented at the A. C. S. Meeting in New York, September, 1954, in the division of Carbohydrate Chemistry. For paper XI of this series *cf.* H. M. Kissman, C. Pidacks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955).

(2) B. R. Baker, J. P. Joseph and J. H. Williams, paper VII of this series, *ibid.*, **77**, 1 (1955).

(3) (a) B. R. Baker, R. E. Schaub and J. H. Williams, paper VIII of this series, *ibid.*, **77**, 7 (1955); (b) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, paper IX of this series, *ibid.*, **77**, 12 (1955).

(4) P. Bennett, S. Halliday and J. J. Oleson, to be published.

(5) R. I. Hewitt, A. Gumble, W. S. Wallace and J. H. Williams, *Antibiotic and Chemotherapy*, **4**, 1222 (1954).

(6) α- and β-ribazole are the only known pair of anomers of a biologically active nucleoside. G. Emerson, F. W. Holly, C. H. Shunk, N. G. Brink, and K. Folkers (THIS JOURNAL, **73**, 1069 (1951)) have shown that both α- and β-ribazole have the same order of vitamin B_{12} activity.

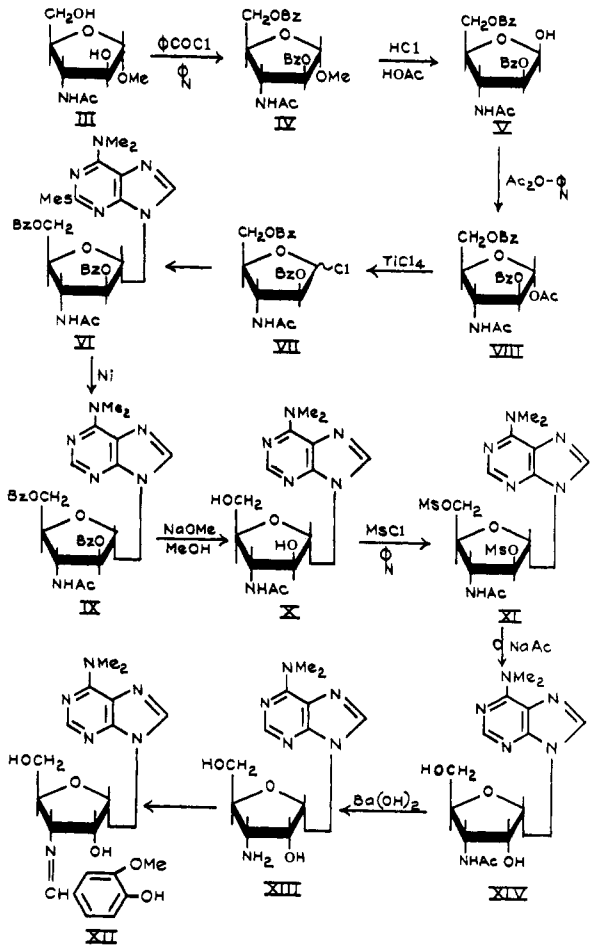
(7) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, paper V of this series, *J. Org. Chem.*, **19**, 1786 (1954).

(8) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).

(9) N. W. Bristow and B. Lythgoe, *J. Chem. Soc.*, 2306 (1949).

(10) B. R. Baker and R. E. Schaub, paper III of this series, *J. Org. Chem.*, **19**, 646 (1954).

(11) B. R. Baker, J. P. Joseph and J. H. Williams, paper IV of this series, *ibid.*, **19**, 1780 (1954).



tion to IX, then hydrolysis back to 2,5-dibenzoyl-3-acetamino-3-deoxy-D-arabinose (V). That this low-melting isomer had the α-configuration was clearly shown by its high positive rotation[12] of $[\alpha]D$ +102°. Therefore, the low-melting isomer was the expected 6-dimethylamino-9-(3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine (X). The high-melting by-product was subsequently found to be identical with the α-ribosylpurine (XIV) which will be discussed in detail later.

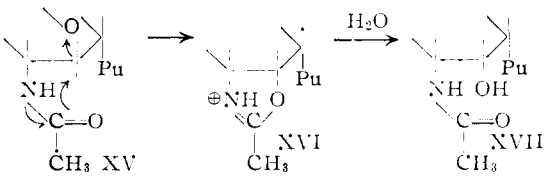
A study of reaction conditions for the sequence VIII → X subsequently showed that the formation of the high-melting by-product could be avoided by reaction of a mixture of VIII and 2-methylmercapto-6-dimethylaminopurine mercuric chloride in ethylene dichloride by addition of titanium tetrachloride. Thus, an over-all yield of 39% of nucleoside X was obtained for the four steps from 1-acetyl-2,5-dibenzoyl-3-acetamino-3-deoxy-D-arabinofuranose (VIII).

Reaction of the α-arabinosylpurine (X) with methanesulfonyl chloride in pyridine at room temperature gave the dimesylate XI as a glass in 85% yield. When XI was refluxed with excess sodium acetate in 95% methyl Cellosolve, the 2′-mesyl group was inverted by attack of the 3′-acetamino group *via* an oxazoline which was hydrolyzed by the water present. At the same time the 5′-mesyl group was replaced by acetate.[2,10] The product was separated from inorganic material by acetylation in pyridine with acetic anhydride to give an 84% yield of the O-diacetate of XIV. O-Deacetylation with methanolic sodium methoxide formed an 82% yield of the desired α-ribosylpurine XIV, m.p. 240°, $[\alpha]D$ +115°, which was identical with the high-melting by-product obtained during formation of X from VIII.[12]

Hydrolysis of the N-acetyl α-aminonucleoside (XIV) with 0.5 *N* barium hydroxide at 100° removed the N-acetyl group with formation of an 80% crude yield of the α-aminonucleoside (XIII) as a glass. This was further purified by conversion to its vanillylidene derivative (XII), m.p. 237°, in 56% yield over-all from XIV.

Biological testing showed that XII had no activity against *Trypanosoma equiperdum* in mice or against the transplanted adenocarcinoma of the C_3H mouse. The vanillylidene derivative of the aminonucleoside (II) was as active as II in these tests. Thus to maintain activity the nucleoside (II) can have only the β-configuration.

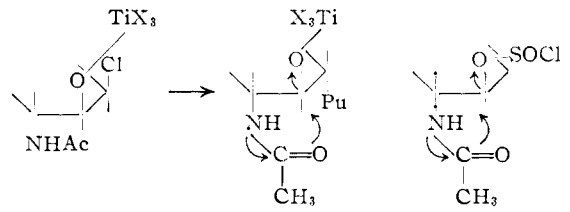
It is interesting that when 1-acetyl-2,5-dibenzoyl-3-acetamino-3-deoxy-D-arabinofuranose (VIII) is treated first with titanium tetrachloride supposedly to form VII, then condensed with the purine, the α-ribosylpurine XIV is a by-product in the sequence for preparation of X. At first inspection this result would appear to contradict the postulated rule of formation of a C_1–C_2-*trans* configuration during nucleoside synthesis from a purine and a chloroacyl sugar.[7] However, it is clear that this inversion at C_2 must have taken place after the nucleoside was formed. If inversion at C_2 had taken place in VII or VIII to give the ribose configuration, then a β-ribosylpurine also should have been obtained.[3] None of the known[3] latter compound could be isolated from the reaction mixture proving that inversion at C_2 took place after condensation to an α-arabinosylpurine. Thus, the inversion at C_2 could take place by attack of the 3′-acetamino group on the 2′-carbon with inversion as shown in the sequence XV → XVI. The intermediate oxazoline XVI then could be hydrolyzed to XVII during work-up.



It is more difficult to explain why reaction of VIII with titanium tetrachloride in the presence of a chloromercury purine does not give any inversion at C_2. It is clear that when the bond of the oxygen at C_2 in XV is connected to benzoyl, no inversion takes place on treatment with titanium tetrachloride. This was confirmed experimentally by O-benzoylation of X, treatment with titanium tetrachloride, then debenzoylation back to X. No α-ribosylpurine XIV was obtained. Therefore it follows that when VIII is treated directly with

(12) The fact that the 5′-mesyl group of XI can be displaced by acetate without quaternization on the N_3-nitrogen proves unequivocally that both X and XIV have the α-configuration (*cf.* B. R. Baker and J. P. Joseph, paper X of this series, THIS JOURNAL, **77**, 15 (1955)).

titanium tetrachloride to form VII, a secondary reaction takes place removing the 2-benzoyl group which allows attack by the 3′-acetamino group only after the purine has been attached to the sugar. A possible type of secondary reaction product would be XVIII where X_3 are halogens and/or benzoate. Once XIX is formed the C_2–O



XVIII XIX XX

linkage is weakened by the electron attraction of titanium which allows inversion to take place by attack of the 3′-acetamino group. A precedent for such a reaction is the inversion of *trans*-2-acetaminocyclohexanol with thionyl chloride[13] *via* a chlorosulfinate (XX). The reason XVIII would not undergo inversion is that the 1-chloro could bond with the titanium, thus causing less weakening of the C_2–O bond. It should be pointed out that experimentally, when titanium tetrachloride is added to a chloromercury purine in ethylene dichloride, the two compounds rapidly complex. Thus there would be less chance for a molecule such as XIX to be formed under these conditions.

Acknowledgment.—The authors are indebted to L. Brancone and staff for the microanalyses and W. Fulmor and staff for the rotations and spectrophotometric data.

Experimental

Methyl 2,5-Dibenzoyl-3-acetamino-3-deoxy-α-D-arabinofuranoside (IV).—To a solution of 9.6 g. of III[2] in 96 cc. of reagent pyridine cooled to 3° in an ice-bath was added 13.6 cc. of benzoyl chloride at such a rate that the temperature was 5–9°. After 3 days at 3° in a stoppered flask, the mixture was poured into 400 cc. of iced water which caused separation of a gum. The mixture was extracted with methylene chloride (3 × 100 cc.). The combined extracts, washed with excess aqueous sodium bicarbonate, were dried with magnesium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in several volumes of toluene and the evaporation *in vacuo* repeated to remove pyridine; yield 21.5 g. (110%) of a sirup which contained some benzoic anhydride and was used in the next step without further purification.

In a pilot run the yield was 6.5 g. (108%) of a gum which could not be crystallized. A sample was dried in high vacuum at 80° for analysis giving a glass with $[\alpha]^{25}D$ +4.0° (1.5% in $CHCl_3$).

Anal. Calcd. for $C_{22}H_{23}NO_7$: C, 64.0; H, 5.57; N, 3.39. Found: C, 64.4; H, 5.80; N, 3.12.

2,5-Dibenzoyl-3-acetamido-3-deoxy-D-arabinose (V).—To a solution of the above 21.5 g. of crude IV in 215 cc. of acetic acid was added 64 cc. of 12 *N* hydrochloric acid. The solution was warmed to 50°, then kept in a 50° bath for 30 minutes. Dilution with 800 cc. of iced water caused an oil to separate which was extracted with chloroform (3 × 150 cc.). The combined extracts, washed with excess aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated *in vacuo* leaving 18.2 g. of a glass. Crystallization from 60 cc. of benzene afforded 8.5 g. (41% based on III) of product, m.p. 152–153°.

The mother liquor was evaporated to dryness *in vacuo* leaving 9.0 g. of residue. This was retreated with 90 cc. of acetic acid and 27 cc. of 12 *N* hydrochloric acid to give an additional 2.8 g. (54% total based on III) of product, m.p. 151–153°.

A similar preparation was recrystallized from benzene–ethyl acetate to give white crystals, m.p. 152–153°, $[\alpha]^{22}D$ −25.6° (2% in $CHCl_3$).

Anal. Calcd. for $C_{21}H_{21}NO_7$: C, 63.1; H, 5.26; N, 3.51. Found: C, 63.1; H, 5.59; N, 3.41.

Methyl 2,5-Dibenzoyl-3-acetamido-3-deoxy-β-D-arabinofuranoside.—Benzoylation of 2.0 g. of methyl 3-acetamido-3-deoxy-β-D-arabinofuranoside[2] as described for the preparation of IV gave 3.9 g. (97%) of crude product, m.p. 137–139°. Recrystallization from ethyl acetate–heptane afforded white crystals, m.p. 152–153°, $[\alpha]^{25}D$ −58.2° (2% in $CHCl_3$).

Anal. Calcd. for $C_{22}H_{23}NO_7$: C, 64.0; H, 5.57; N, 3.39. Found: C, 64.3; H, 5.92; N, 3.34.

Hydrolysis of the crude methyl ether as described for IV gave a 55% yield of V, m.p. 151–153°.

1-Acetyl-2,5-dibenzoyl-3-acetamido-3-deoxy-α-D-arabinofuranoside (VIII).—A solution of 4.0 g. of V in 20 cc. of reagent pyridine and 20 cc. of acetic anhydride was heated on the steam-bath for 1 hour. Dilution with 100 cc. of iced water caused a gum to separate which was extracted with chloroform (3 × 25 cc.). Dried with magnesium sulfate, the combined extracts were evaporated to dryness *in vacuo*. The residue was dissolved in several volumes of toluene, then evaporated to dryness *in vacuo* to remove pyridine leaving 4.8 g. (108%) of a sirup. Crystallization from benzene–heptane afforded 3.3 g. (75%) of one crystalline anomer, m.p. 119–121°, which was suitable for the next step.

Recrystallization of a similar preparation from benzene–heptane gave white crystals, m.p. 121–122°, $[\alpha]^{24}D$ +29.1° (2% in $CHCl_3$).

Anal. Calcd. for $C_{23}H_{23}NO_8$: C, 62.6; H, 5.21; N, 3.18. Found: C, 62.4; H, 5.26; N, 3.10.

2-Methylmercapto-6-dimethylamino-9-(2′,5′-dibenzoyl-3′-acetamido-3′-deoxy-α-D-arabinofuranosyl)-purine (VI) (A).—A mixture of 4.55 g. of 2-methylmercapto-6-dimethylaminopurine mercuric chloride,[11] 5.0 g. of Celite, 3.6 g. of VIII and 325 cc. of ethylene dichloride was freed from traces of water by distillation of 25 cc. of solvent. To the somewhat cooled, stirred mixture was added a solution of 1.1 cc. of titanium tetrachloride in 15 cc. of ethylene chloride over a period of about 2 minutes. The chloromercury purine rapidly dissolved to a light-brown solution. In a few minutes the color began to get lighter due to separation of a purine titanium complex as a fine precipitate. The mixture was refluxed and stirred for 21 hours, then diluted with 150 cc. of water and stirred for 15 minutes without further heating. The mixture was filtered and the filter-cake washed with hot chloroform. The organic layer was separated from the combined filtrate and washings, then evaporated to dryness *in vacuo*. A solution of the residue in 50 cc. of chloroform was filtered to remove a small amount of insoluble tar, then washed with 50 cc. of 30% aqueous potassium iodide followed by water. After being dried with magnesium sulfate, the solution was evaporated to dryness *in vacuo* leaving 4.7 g. (98%) of a glass which by ultraviolet analysis contained a maximum of 89% of a nucleoside with the mol. wt. of VI.

(B).—To a solution of 3.3 g. of VIII in 28 cc. of ethylene dichloride was added a solution of 1.0 cc. of titanium tetrachloride in 15 cc. of ethylene dichloride. A yellow precipitate separated which rapidly redissolved when the mixture was shaken. The solution was refluxed on the steam-bath protected from moisture for 1 hour during which time it became brown. This solution was added to a stirred and previously distillation-dried mixture of 4.15 g. of 2-methylmercapto-6-dimethylaminopurine mercuric chloride,[11] 4.5 g. of Celite and 300 cc. of ethylene dichloride. Again as in A the purine dissolved and soon reprecipitated as a titanium complex. The reaction mixture was processed as in A to give 3.8 g. (86%) of a glass which by ultraviolet analysis contained 110% of a nucleoside with the molecular weight of VII. Apparently some O-benzoyl (see Discussion) was lost which would account for this discrepancy.

6-Dimethylamino-9-(2′,5′-dibenzoyl-3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine (IX). (A).—A solution of 4.7 g. of crude VI, prepared by method A, in 250 cc. of methyl Cellosolve was stirred on the steam-bath with about

(13) W. S. Johnson and E. N. Schubert, THIS JOURNAL, **72**, 2187 (1950); G. E. McCasland and D. A. Smith, *ibid.*, **72**, 2190 (1950).

5 teaspoons of desulfurizing Raney nickel[14] for 35 minutes. The hot mixture was filtered through Celite. The combined filtrate and washings were evaporated to dryness *in vacuo* leaving 3.11 g. (72%) of product, which by ultraviolet analysis contained 100% of a nucleoside with the mol. wt. of IX.

(B).—Desulfurization of 3.8 g. of VI, prepared by method B, gave 2.5 g. (71%) of product which by ultraviolet analysis contained 90% of a nucleoside with the molecular weight of IX.

6-Dimethylamino-9-(3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine (X). (A).—To a solution of 3.11 g. of crude IX, prepared by method A, in 67 cc. of methanol, was added 1.2 cc. of 1 *N* methanolic sodium methoxide. The solution was refluxed for 35 minutes with the *p*H remaining > 10 when spotted on moist indicator paper.[15] Evaporation *in vacuo* left a glass which was triturated with hot ethyl acetate. The solid (3.26 g.) was collected and treated below. The filtrate deposited 372 mg. (19%) of product, m.p. 188–190°, on standing for a few days. The 3.26 g. of ethyl acetate insoluble material was ground in a mortar with 4 g. of sand, then extracted in a Soxhlet with boiling ethyl acetate until no more product could be obtained (20–40 hours). Concentration of the extract gave 701 mg. of product in several crops, m.p. between 182 and 191°, suitable for the next step. The total yield was 1.073 g. (39% over-all from VIII). A sample was dissolved in hot ethyl acetate by the addition of sufficient absolute alcohol to cause solution, then crystallized by the addition of heptane to turbidity; white crystals, m.p. 189–191°, $[\alpha]^{25}D$ +102° (1.7% in H_2O). This compound had the typical ultraviolet spectrum of a 9-substituted-6-dimethylaminopurine.[11,16] In the infrared the compound showed OH–NH absorption at 2.94 and 3.15 μ, –CONH– absorption at 5.94 μ and C=N absorption at 6.17 μ.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 6.00; N, 25.0. Found: C, 49.9; H, 6.13; N, 24.9.

(B).—Debenzoylation of 2.5 g. of crude IX, prepared by method B, as described in method A gave 1.39 g. of ethyl acetate insoluble material. The ethyl acetate solution deposited 95 mg. (6%) of X, m.p. 189–191°, which was identical with preparation A. The 1.39 g. of ethyl acetate insoluble material was continuously extracted in a Soxhlet apparatus with ethyl acetate for 3 hours. On cooling 445 mg. (29%) of solid was collected, m.p. 195–197°, which proved to be a mixture of X and XIV. A second crop of 18 mg. (1%) of nearly pure XIV, m.p. 228–234°, was obtained.

Recrystallization of 330 mg. of the 445 mg. of product as described for X (procedure A) gave 60 mg. of XIV, m.p. 235–236°, $[\alpha]^{25}D$ +114° (0.5% in H_2O). No further attempt was made to separate these isomers.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 6.00; N, 25.0. Found: C, 49.7; H, 6.46; N, 24.3.

This compound had the typical ultraviolet spectrum of a 9-substituted-6-dimethylaminopurine[11,16] and in the infrared showed OH–NH absorption at 2.93, 3.02 and 3.15 μ, amide absorption at 5.99 μ and C=N absorption at 6.18 μ. The general spectrum was considerably different from that of X. The structure of this compound was unknown at this time, but subsequently proved to be identical with XIV.

6-Dimethylamino-9-(2′,5′-dimesyl-3′-acetamino-3′-deoxy-α-D-arabinofuranosyl)-purine (XI).—To a solution of 500 mg. of X in 10 cc. of reagent pyridine cooled in an ice-bath was added 0.50 cc. of methanesulfonyl chloride. The solution was allowed to stand in a stoppered flask at room temperature for 48 hours. After dilution with 50 cc. of iced water, the mixture was extracted with chloroform (7 × 25 cc.). The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 622 mg. (85%) of a glass which did not crystallize and was not quite pure.

Anal. Calcd. for $C_{16}H_{24}N_6O_8S_2$: C, 39.0; H, 4.90; N, 17.1. Found: C, 39.1; H, 5.69; N, 16.3.

6-Dimethylamino-9-(2′,5′-diacetyl-3′-acetamino-3′-deoxy-α-D-ribofuranosyl)-purine.—A mixture of 602 mg. of XI, 0.50 g. of anhydrous sodium acetate and 5.3 cc. of methyl Cellosolve containing 5% water was refluxed for 24 hours, solution being complete at the boiling point. During this time sodium methanesulfonate separated. The cooled solution was filtered from the sodium methanesulfonate (160 mg.) and evaporated to dryness *in vacuo*. The residue was heated with 4 cc. of reagent pyridine and 4 cc. of acetic anhydride on the steam-bath for 1 hour. After dilution with 20 cc. of iced water the solution was extracted with chloroform (3 × 15 cc.). The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 450 mg. (84%) of a glass which could not be crystallized.

Anal. Calcd. for $C_{18}H_{24}N_6O_6$: C, 51.4; H, 5.75; N, 20.0. Found: C, 51.4; H, 6.12; N, 19.4.

6-Dimethylamino-9-(3′-acetamino-3′-deoxy-α-D-ribofuranosyl)-purine (XIV).—To a solution of 400 mg. of the preceding compound in 8 cc. of dry methanol was added 0.18 cc. of 1 *N* methanolic sodium methoxide. After being refluxed for 30 minutes,[15] the solution was evaporated to dryness *in vacuo*. Trituration of the glassy residue with 3 cc. of ethyl acetate caused crystallization; yield 250 mg. (82%), m.p. 233–235° dec. Recrystallization from methanol gave white crystals, m.p. 239–240°, $[\alpha]^{25}D$ +115° (0.5% in H_2O), $\lambda_{max}^{pH\ 1}$ 268 mμ (ε 19,600), $\lambda_{max}^{pH\ 7}$ 275 mμ (ε 20,000), $\lambda_{max}^{pH\ 14}$ 275 mμ (ε 19,800).[11,16] The over-all yield from X was 58%.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 6.00; N, 25.0. Found: C, 49.0; H, 5.91; N, 25.1.

This compound gave no depression in m.p. when mixed with the by-product formed in the preparation of X (method B) and both had identical infrared spectra.

6-Dimethylamino-9-(3′-vanillylideneamino-3′-deoxy-α-D-ribofuranosyl)-purine (XII).—A solution of 850 mg. of XIV in 40 cc. of 0.5 *N* barium hydroxide was heated on the steam-bath for 30 minutes. The cooled solution was treated with Dry Ice until no more barium carbonate was precipitated. The filtered solution was evaporated to dryness *in vacuo*. The residual crude XIII, containing some XIV, was dissolved in 25 cc. of 75% alcohol and 580 mg. of vanillin was added. After being refluxed for 1 hour, the solution was evaporated to dryness *in vacuo*. The residue was shaken with 20 cc. of ethyl acetate and 20 cc. of water. The product which separated at the interphase was collected by filtration and washed with water and ethyl acetate; yield 285 mg. (27.4%), m.p. 234–235°. A mixture with XIV melted at 219–220°. The ethyl acetate layer in the filtrate was evaporated *in vacuo*. Crystallization from ether gave 50 mg. (4.6%) of crude product, m.p. 212–218°.

The aqueous layer was evaporated to dryness *in vacuo* and retreated with barium hydroxide, then vanillin as above to give an additional 190 mg. (17.6%) of product, m.p. 234–235°. A third treatment with barium hydroxide formed an additional 65 mg. (6.0%) of product, m.p. 236–237°. The total yield was 605 mg. (56%): all fractions were identical with the first crop as shown by mixed m.p. data. A sample of the first crop was recrystallized from 50% methyl Cellosolve to give white crystals. m.p. 237–237.5°, $[\alpha]^{25}D$ −45° (1.5% in pyridine).

Anal. Calcd. for $C_{20}H_{24}N_6O_5$: C, 56.1; H, 5.65; N, 19.6. Found: C, 55.8; H, 5.98; N, 19.8.

This compound showed OH absorption at 3.12 μ, C=N (from anil) at 6.04 μ and C=N (from purine) at 6.18 μ in the infrared.

The intermediate XIII can be isolated as a glass after a 2-hour hydrolysis as hygroscopic crystals, m.p. 235° dec., in 80% yield, but it cannot be purified as such. The infrared spectrum showed the loss of the –CONH– band characteristic of XIV.

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(14) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers. THIS JOURNAL, **65**, 1013 (1943).

(15) If the solution does not remain this basic, additional portions of 1 *N* methanolic sodium methoxide should be added until the solution maintains *p*H >10 for a total of 30 minutes reflux.

(16) B. R. Baker, R. E. Schaub and J. P. Joseph, Paper II of this series, *J. Org. Chem.*, **19**, 638 (1954).