Anal. Calcd. for $C_8H_{11}O_4N \cdot HCl$: C, 32.4; H, 6.52; N, 7.55. Found: C, 32.4; H, 6.52; N, 7.80.

Methyl 3-Phthalimido-3-deoxy-β-D-xylofuranoside (XXIV, C).—A solution of 4.33 g. of residual gum B (mainly methyl 3-amino-3-deoxy-β-D-xylofuranoside, XVIIb) and 4.33 g. of phthalic anhydride in 43 cc. of dimethylformamide was refluxed for 3 hours and then evaporated to dryness *in vacuo*. The residue was dissolved in 20 cc. of water and the solution saturated with sodium chloride and extracted with three 20-cc. portions of chloroform. The combined chloroform extracts were washed with excess saturated aqueous sodium bicarbonate, dried with magnesium sulfate and evaporated to dryness *in vacuo* to give 6 g. of a sirup. Crystallization from ethyl acetate–heptane afforded 3.35 g. (43%) of product which was obtained in two crops, m.p. 139–142°. When the reaction time was 90 minutes instead of 3 hours as above, the yield was 41% (300 mg.), m.p. 140–142°. In a pilot run, when the reaction time was 45 minutes, the yield was 20% (100 mg.), m.p. 140–142°. Recrystallization from ethyl acetate–heptane afforded white crystals, m.p. 140–142°, $[\alpha]^{25}D$ +90° (2% in $CHCl_3$); λ_{max}^{KBr} 2.92 μ (OH); 5.67, 5.85 μ (C=O of phthalimido).

Anal. Calcd. for $C_{14}H_{15}O_6N$: C, 57.4; H, 5.15; N, 4.78. Found: C, 57.2; H, 5.53; N, 5.05.

Methyl 3-Phthalimido-3-deoxy-α-D-xylofuranoside.—By refluxing a solution of 75 mg. of crystalline methyl 3-amino-3-deoxy-α-D-xylofuranoside (XVIIa) and 75 mg. of phthalic anhydride in 3 cc. of dimethylformamide for 90 minutes, there was obtained, as described above for the preparation of XXIV, 37 mg. (27%) of product, m.p. 126–128°. Recrystallization from ethyl acetate–heptane afforded white crystals, m.p. 128–129°, $[\alpha]^{25}D$ +231° (0.4% in $CHCl_3$); λ_{max}^{KBr} 2.92 μ (OH); 5.63, 5.84 μ (C=O of phthalimido).

Anal. Calcd. for $C_{14}H_{15}O_6N$: C, 57.4; H, 5.15; N, 4.78. Found: C, 57.1; H, 5.39; N, 4.95.

Methyl 3-Acetamido-3-deoxy-β-D-xylofuranoside.—To a solution of 323 mg. of gum B (mainly methyl 3-amino-3-deoxy-β-D-xylofuranoside, XVIIb) in 2 cc. of water was added 0.3 cc. of acetic anhydride. The mixture was shaken for 7 minutes, then evaporated to dryness *in vacuo*. Crystallization of the residual sirup from ethyl acetate–absolute alcohol afforded 183 mg. (45%) of product which was obtained in two crops, m.p. 107–109°. Recrystallization from ethyl acetate–absolute alcohol gave white crystals, m.p. 109–110°, $[\alpha]^{25}D$ −30.7° (2% in H_2O); λ_{max}^{KBr} 3.05, 3.41 μ (OH and NH); 6.07, 6.48 μ (secondary amide); 8.81, 9.1, 9.5 μ (C–O–C and C–OH). This compound failed to consume any periodate in aqueous sodium bicarbonate after 30 minutes.

Anal. Calcd. for $C_8H_{15}O_5N$: C, 46.8; H, 7.36; N, 6.83. Found: C, 46.9; H, 7.61; N, 6.88.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, PEARL RIVER LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

The Synthesis of 9-(3-Amino-3-deoxy-β-D-xylofuranosyl)-6-dimethylaminopurine, an Analog of the Aminonucleoside Derived from Puromycin

BY ROBERT E. SCHAUB, MARTIN J. WEISS AND B. R. BAKER

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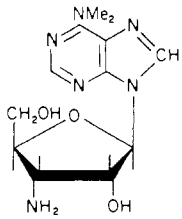
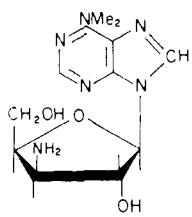
The subject nucleoside II was prepared by condensation of 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-xylofuranosyl chloride (XVIII) with 6-chloropurine-mercuri chloride followed by dimethylamine and butylamine treatment. Halogenose XVIII was prepared from methyl 3-phthalimido-3-deoxy-β-D-xylofuranoside (XV) by 2,5-di-*O*-benzoylation, acetolysis of the glycosidic linkage and, finally, ethereal hydrogen chloride treatment. Attempts to prepare II *via* the reaction of ammonia with 9-(2,3-anhydro-β-D-ribofuranosyl)-6-dimethylamino-2-methylmercaptopurine (VIII) or with the corresponding 5'-*O*-trityl derivative III failed. The synthesis of the 2,3-anhydroribofuranosyl nucleosides III and VIII are described.

A pertinent analog of the aminonucleoside I derived from the antibiotic puromycin[1] is 9-(3-amino-3-deoxy-β-D-xylofuranosyl)-6-dimethylaminopurine (II). Analogs of I are of interest because of the carcinostatic[2] and trypanocidal[3] activities in experimental animals which this compound exhibits. This paper describes the synthesis of analog II.

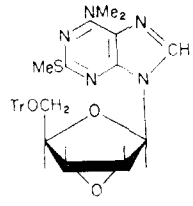


I



II

Previous work from this Laboratory has shown that aminonucleosides can be prepared by the amination of anhydronucleosides. Thus, a 3-aminoarabinoside was prepared by reaction of the corresponding 2,3-anhydrolyxofuranosylpurine with ammonia.[4] By analogy, it was expected that the synthesis of II would proceed from the reaction of ammonia with a 2,3-anhydroribofuranosylpurine, such as III. The reaction of ammonia with the epoxide group of III could conceivably take place at C-2 as well as at the required position, C-3. However, inasmuch as the related anhydronucleoside 7-(2,3-anhydro-5-*O*-trityl-β-D-ribofuranosyl)-theophylline already had been shown[5] to react in high yield with sodium ethyl mercaptide at C-3, it was reasonable to expect that III would react with ammonia also at C-3 to give the desired 3'-aminoxyloside.



III

(1) For the chemistry of puromycin see B. R. Baker and co-workers, THIS JOURNAL, **77**, 12 (1955), and preceding papers.

(2) P. L. Bennett, S. L. Halliday, J. J. Oleson and J. H. Williams, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, N. Y., 1954, pp. 766–769.

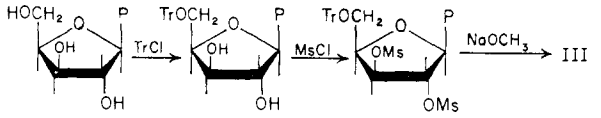
(3) R. I. Hewitt, A. R. Gumble, W. S. Wallace and J. H. Williams, *Antibiotics & Chemotherapy*, **4**, 1222 (1954).

(4) B. R. Baker and R. E. Schaub, THIS JOURNAL, **77**, 5900 (1955).

(5) J. Davoll, B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 2230 (1951).

Anhydronucleoside III was prepared in 35% overall yield from the known[4] 6-dimethylamino-2-methylmercapto-9-β-D-xylofuranosylpurine (IV)[6] as follows. Reaction of IV with triphenylmethyl (trityl) chloride in pyridine gave the crude 5′-*O*-trityl derivative V, treatment of which with methanesulfonyl (mesyl) chloride in pyridine solution produced a 96% yield of the amorphous dimesylate VI. Methanolic sodium methoxide treatment of VI then afforded the crystalline anhydronucleoside III.[5,7-9]

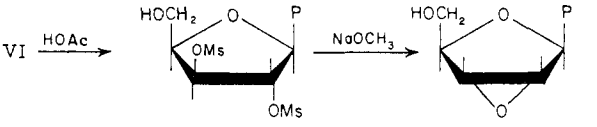
The formation of an epoxy ring presumably involves acyl–oxygen cleavage of the 2-*O*-sulfonyl group to produce an oxygen anion which then displaces the adjacent *trans*-3-sulfonyloxy group by Walden inversion.[7] With VI, such a process would give the 2,3-anhydroriboside (III). The reverse possibility, acyl–oxygen cleavage of the 3-*O*-mesyl group and displacement of the 2-mesyloxy group, would produce the corresponding 2,3-anhydrolyxoside. Confirmation of the postulated structure III was obtained by an unequivocal synthesis in 71% yield from the 2-*O*-benzoyl-3-*O*-mesyl-5-*O*-tritylxyloside (XIII) (see below)



IV V VI

P = 2-methylmercapto-6-dimethylaminopurinyl-9

All attempts to effect the amination of anhydronucleoside III were unsuccessful, and either starting material or unworkable tars were obtained. The failure of III to react with ammonia to give an aminonucleoside was conceivably the result of a blocking effect exerted by the bulky trityl and purinyl groups. Hence, it was decided to investigate the reaction of ammonia with the non-tritylated and, therefore, presumably less-hindered anhydronucleoside VIII. This anhydronucleoside VIII was obtained as a crystalline product in two steps in 19% over-all yield from the 2′,3′-di-*O*-mesyl-5′-*O*-tritylnucleoside (VI) by detritylation (to give 56% crystalline VII) followed by epoxide ring closure with methanolic methoxide (34% yield). An attempt to prepare VIII directly by detritylation of the 2′,3′-anhydro-5′-*O*-trityl nucleoside (III) was unsuccessful.



VII VIII

P = 6-dimethylamino-2-methylmercaptopurinyl-9

An alternate synthesis of VIII from the xylofuranosylnucleoside (IV) required seven steps and proceeded in 20% over-all yield.[11] Benzoylation of the known[4] 3′,5′-*O*-isopropylidene derivative IX, obtained in 90% crude yield from IV, quantitatively gave the amorphous 2′-*O*-benzoyl-3′,5′-*O*-isopropylidene xyloside (X), which on deacetonation with 70% aqueous acetic acid afforded the crystalline 2′-*O*-benzoate XI in 49% yield. This 2′-*O*-benzoate was tritylated to produce in quantitative yield the amorphous 2′-*O*-benzoyl-5′-*O*-trityl nucleoside XII which was converted to the 3′-*O*-mesyl derivative XIII, obtained quantitatively as a glass. Detritylation of XIII with 80% aqueous acetic acid afforded the non-crystalline 2′-*O*-benzoyl-3′-*O*-mesyl nucleoside XIV in 88% crude yield. Finally, overnight treatment of crude XIV with methanolic sodium methoxide at 5° resulted in epoxide ring closure and the formation in 52% yield of the required VIII, crystalline after three amorphous intermediates. This product was identical with the material synthesized from dimesylate VII. Confirmation of assigned structure VIII was obtained by tritylation in 12% yield to the previously prepared 2′,3′-anhydro-5′-*O*-trityl nucleoside III.[12]

Intensive efforts to effect the amination of VIII with methanolic or aqueous ammonia at temperatures ranging from 100 to 200° were all unsuccessful. Either starting material or gums which did not contain any appreciable amount of an acid-soluble fraction were obtained. The inability of ammonia to react with anhydronucleoside VIII or the 5′-*O*-trityl-anhydronucleoside III is surprising in view of the successful reaction[5] of sodium ethyl mercaptide with 7-(2,3-anhydro-5-*O*-trityl-β-D-ribofuranosyl)-theophylline. The apparently greater reactivity of mercaptide anion as compared to ammonia is probably attributable to the increased nucleophilicity of the former reagent.[13,14]

An obvious alternative approach to the synthesis of II involved the preparation of an alkyl 3-aminoxylofuranoside by the reaction of ammonia with a 2,3-anhydroribofuranosyl glycoside, which in the absence of the bulky purinyl group would be expected to be more susceptible to ammonia attack.

(6) The 6-dimethylamino-2-methylmercapto nucleoside was used rather than the 6-dimethylamino nucleoside itself since Baker and Schaub[4] have shown that condensation of 6-dimethylaminopurine mercuric chloride with 2,3,5-tri-*O*-benzoyl-D-xylofuranosyl bromide gives a mixture of 7- and 9-xylosides. However, condensation of this sugar bromide with 6-dimethylamino-2-methylmercaptopurine mercuric chloride proceeds exclusively to the 9-xyloside.[4] The 2-methylmercapto group is removable by Raney nickel desulfurization.[4]

(7) G. R. Robertson and C. F. Griffith, *J. Chem. Soc.*, 1193 (1935).

(8) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **63**, 1727 (1941).

(9) The literature examples involved the use of 2,3-di-*O*-tosylates and with these compounds ring closure was achieved by a relatively mild methoxide treatment (0°, 3 days).[5,8] These conditions, however, failed with dimesylate VI and 80% of the starting material was recovered. The more vigorous conditions (two hours in refluxing methanolic methoxide solution) which finally were used to effect epoxide formation gave lower yields. Presumably, the lower yields resulted from a subsequent reaction of methoxide with the newly formed epoxide.[7] In this respect, it is to be noted that the milder methoxide treatment (5°, overnight) of the 2′-*O*-benzoyl-3′-*O*-mesyl nucleoside XIV gave a 52% yield of anhydronucleoside VIII. Similar results have been obtained with the 2,3-di-*O*-mesyl- and the 2-*O*-benzoyl-3-*O*-mesylxyloside derivatives in the methyl glycoside series.[10]

(10) R. E. Schaub and M. J. Weiss, THIS JOURNAL, **80**, 4683 (1958).

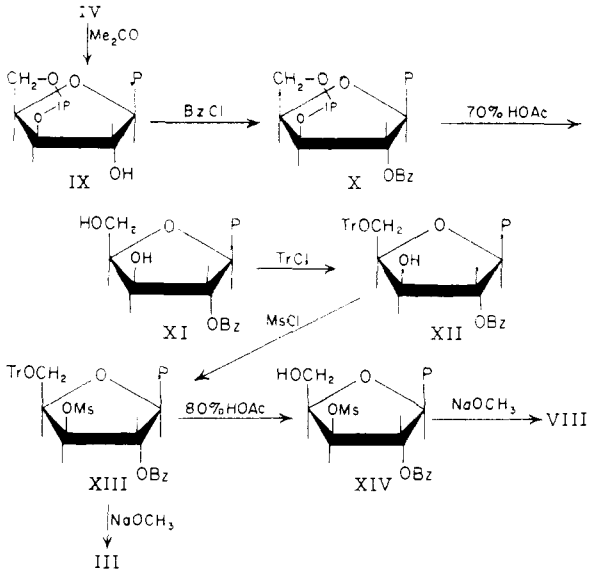
(11) For comparison, the synthesis of VIII from IV *via* dimesylate VII required four steps and proceeded in 17% over-all yield.

(12) The possibility existed that the product considered to have structure VIII was, in actual fact, a 2,5-anhydroarabinoside or a 3,5-anhydroxyloside. Such products could conceivably result from an intramolecular attack of the 5′-hydroxy group on the initially formed 2′,3′-epoxy group during the treatment of VII or XIV with methanolic methoxide.

(13) See J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, pp. 138–142.

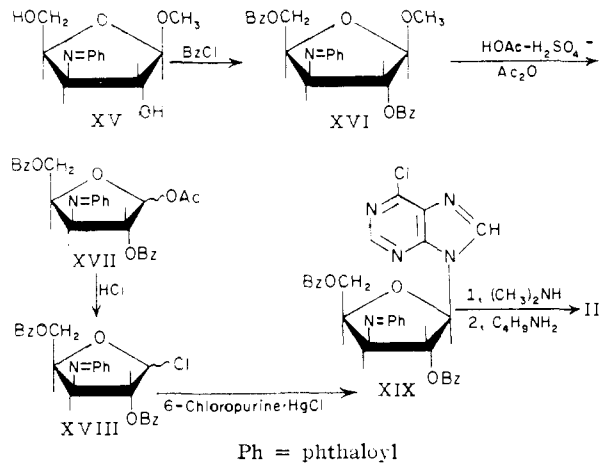
(14) J. F. Bunnett and G. T. Davis, THIS JOURNAL, **76**, 3011 (1954).

The successful preparation of an alkyl 3-aminoxylofuranoside would then, in principle, lead to a synthesis of the desired nucleoside II *via* the conversion of the aminoxyloside to a properly blocked 1-chloroxylose derivative, condensation of this halogenose with the mercuric chloride derivative of 6-chloropurine, replacement of the 6-chlorine of the resulting blocked nucleoside with dimethylamine and, finally, complete deblocking.



The synthesis of the required 3-aminoxylofuranoside, namely, methyl 3-phthalimido-3-deoxy-β-D-xylofuranoside (XV), is described in an accompanying paper.[10] Benzoylation of XV gave a quantitative yield of the amorphous 2,5-di-*O*-benzoyl derivative XVI, acetolysis of which with acetic anhydride–acetic acid–sulfuric acid[15] afforded a 72% yield of a crystalline 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-phthalimido derivative XVII ($[\alpha]_D$ +79°) of unknown anomeric identity.[16] Treatment of the 1-*O*-acetate (XVII) with ethereal hydrogen chloride quantitatively gave a gum which was presumably an anomeric mixture of the blocked 1-chloro sugar XVIII.[15]

Condensation of this anomeric mixture of halogenoses (XVIII) with 6-chloropurine mercuric chloride derivative[17,18] in refluxing xylene gave the crystalline blocked 6-chloronucleoside XIX in 47% yield. Reaction of XIX with methanolic dimethylamine at 100° for two hours followed by treatment with methanolic butylamine at reflux for eighteen hours, afforded the desired 9-(3-amino-3-deoxy-β-D-xylofuranosyl)-6-dimethylaminopurine (II) as



a glass in 80% yield.[19] Slow evaporation at room temperature of a solution of this glass in ethyl acetate–ethanol gave a crystalline product, m.p. 147–149°, in 62% yield (from XIX). A crystalline vanillylidene derivative was obtained in 96% yield from crystalline nucleoside and in 83% yield from the crude glass.

It was not feasible to prove unequivocally the assigned β-configuration for II.[20] However, the assumption of a β-configuration for this product is reasonable since condensation of a 1-chloro-2-acyloxy sugar with a purine mercuric chloride derivative, to our knowledge, always has given, at least as a major product, a nucleoside wherein the purine is *trans* to the 2-acyloxy group ("C_1–C_2-*trans* rule").[23] Stereospecificity presumably results from neighboring group participation of the 2-acyloxy group *via* an intermediate *ortho* ester cation.[24] Although α-nucleosides have been obtained, in violation of the "C_1–C_2-*trans* rule," from the condensation of 1-chloro-2-acyloxy sugars with purine or benzimidazole mercuric chloride derivatives, the yields have been low and the α-nucleoside has always been accompanied by substantially larger amounts of β-anomer.[18,25–27] In view of this general experience, the relatively high (for nucleoside condensations) yield of 47% for crystalline XIX in-

(15) B. R. Baker, J. P. Joseph and R. E. Schaub, THIS JOURNAL, **77**, 5905 (1955).

(16) Usually a specific rotation as high as +79° might be taken to indicate an α-anomer. However, the β-anomer of methyl 3-phthalimido-3-deoxy-D-xylofuranoside has the high specific rotation of +90° and the α-anomer has a specific rotation of +231°.[10]

(17) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(18) L. Goldman, J. W. Marsico and R. B. Angier, THIS JOURNAL, **78**, 4173 (1956).

(19) Methanolic dimethylamine treatment, besides introducing the 6-dimethylamino-group, usually results in the removal of *O*-benzoyl groups and the opening of the phthalimido ring to a phthalamide. Complete deblocking is then achieved by butylamine treatment.[18]

(20) The most convenient procedure for determining the anomeric configuration of a nucleoside is its conversion by periodate oxidation to a bis-aldehyde of a proven structure. Comparison then of the molar rotation values usually suffices to establish configuration.[21] Since II on periodate oxidation would normally be expected to give the same bis-aldehyde as is obtained on oxidation of the aminonucleoside I, unequivocally established[22] as a β-nucleoside; the anomeric configuration of II was theoretically determinable. However, compound I consumed two equivalents of periodate within 24 hours, whereas II took up only 1.6 equivalents after six days. Since I and II did not both give the same product quantitatively, this procedure was inapplicable to this case.[25]

(21) A. R. Todd and co-workers, *J. Chem. Soc.*, 833 (1946); 1613 (1949).

(22) B. R. Baker and J. P. Joseph, THIS JOURNAL, **77**, 15 (1955).

(23) B. R. Baker and R. E. Schaub, *ibid.*, **77**, 2396 (1955).

(24) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

(25) H. M. Kissman, R. G. Child and M. J. Weiss, THIS JOURNAL, **79**, 1185 (1957).

(26) B. R. Baker, R. E. Schaub and H. M. Kissman, *ibid.*, **77**, 5911 (1955).

(27) H. M. Kissman and B. R. Baker, *ibid.*, **79**, 5531 (1957).

dicates that the "C_1–C_2-*trans* rule" was operative during the synthesis of this nucleoside. Furthermore, the specific rotation of −33° is not unreasonable for a β-nucleoside.

Periodate titration of II showed a rapid (seventeen minutes) uptake of 1.0 equivalent of oxidant followed by a slow consumption, which in six days amounted to an additional 0.6 equivalent.[28] This further confirmed the assigned furanoside ring structure for the sugar portion of II.[10] Finally, dilute hydrochloric acid hydrolysis of II gave, in 66% yield, 3-amino-3-deoxy-D-xylose hydrochloride.[10]

On testing against a transplanted mammary adenocarcinoma of the C3H mouse, compound II was essentially inactive.[29]

Acknowledgment.—We wish to thank Mr. L. Brancone and staff for microanalytical data and Mr. W. Fulmor and staff for spectroscopic and polarimetric data.

Experimental[30]

6-Dimethylamino-2-methylmercapto-9-(5-*O*-trityl-β-D-xylofuranosyl)-purine (V).—A solution of 2 g. of 6-dimethylamino-2-methylmercapto-9-β-D-xylofuranosylpurine (IV)[4] and 1.8 g. of triphenylmethyl chloride (10% excess) in 10 cc. of reagent pyridine (protected from atmospheric moisture) was heated in an oil-bath at 51° for 72 hours. The cooled solution was diluted with 30 cc. of chloroform, then 60 cc. of water. Solid sodium bicarbonate was added until the mixture was slightly basic. The separated organic phase was dried with magnesium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in toluene and the evaporation repeated leaving 3.4 g. (100%) of product as a glass. The combustion analysis indicates contamination with about 13% of triphenylcarbinol.

Anal. Calcd. for $C_{32}H_{33}O_4N_5S$: C, 65.9; H, 5.70; N, 12.0. Found: C, 68.3; H, 5.99; N, 10.5.

6-Dimethylamino-9-(2,3-di-*O*-mesyl-5-*O*-trityl-β-D-xylofuranosyl)-2-methylmercaptopurine (VI).—To a solution of 2 g. of 6-dimethylamino-2-methylmercapto-9-(5-*O*-trityl-β-D-xylofuranosyl)-purine (V) in 40 cc. of reagent pyridine, cooled in an ice-bath to 3°, was added 2 cc. of methanesulfonyl chloride at such a rate that the temperature was maintained in the range of 5–9°. The solution was allowed to stand in a stoppered flask at room temperature for 24–72 hours. The cooled solution was diluted with 50 cc. of chloroform, and then 200 cc. of water. Solid sodium bicarbonate was added until the mixture was slightly basic. The separated organic phase was dried with magnesium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in toluene and the evaporation repeated leaving 2.4 g. (95%) of product as a glass which probably contained some triphenylcarbinol.

9-(2,3-Anhydro-5-*O*-trityl-β-D-ribofuranosyl)-6-dimethylamino-2-methylmercaptopurine (III). A. From Dimesylate VI.—A mixture of 2 g. of 6-dimethylamino-9-(2,3-di-*O*-mesyl-5-*O*-trityl-β-D-xylofuranosyl)-2-methylmercaptopurine (VI), 22 cc. of absolute methanol and 11 cc. of 1 *N* methanolic sodium methoxide was heated at reflux with stirring for 2 hours. Solution was complete on reaching the boiling point. During the reflux period a solid separated. The cooled mixture was filtered. The solids were washed with methanol and then several times with water to remove sodium methanesulfonate, leaving 545 mg. (36%) of product, m.p. 211–212° dec.

In a pilot run the yield was 520 mg. (34%), m.p. 208–209° dec. Recrystallization from ethyl acetate–heptane gave white crystals, m.p. 213° dec., $[\alpha]^{25}D$ +68.7° (2.4% in $CHCl_3$); λ_{max}^{KBr} 6.25 μ (C=N); 9.30, 9.47 μ (C–O–C); 14.1, 14.3 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{33}H_{31}O_3N_5S$: C, 68.0; H, 5.52; N, 12.4. Found: C, 67.7; H, 5.58; N, 12.7.

B. From the 2'-*O*-Benzoyl-3'-*O*-mesyl-5'-*O*-trityl Nucleoside XIII.—To a hot solution of 200 mg. of 9-(2-*O*-benzoyl-3-*O*-mesyl-5-*O*-trityl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XIII) (see below) in 5 cc. of absolute methanol was added 0.79 cc. of 1 *N* methanolic sodium methoxide. Crystals began to separate on slight cooling. After 24 hours at 3°, the crystalline solid was collected, washed with water and then with absolute methanol; yield 105 mg. (71%), m.p. and mixed m.p. with the product from preparation A, 215° dec.

C. By Tritylation of Anhydronucleoside VIII.—A solution of 164 mg. of 9-(2,3-anhydro-β-D-ribofuranosyl)-6-dimethylamino-2-methylmercaptopurine (VIII) (see below) and 156 mg. of triphenylmethyl chloride in 2 cc. of reagent pyridine (protected from atmospheric moisture) was heated in an oil-bath at 50° for 72 hours. The cooled solution was diluted with 5 cc. of chloroform, followed by 10 cc. of water. Solid sodium bicarbonate was added until the mixture was slightly basic. The separated organic phase was dried with magnesium sulfate and evaporated to dryness *in vacuo* leaving 290 mg. of glass. Crystallization from ethyl acetate–heptane gave 35 mg. (12%) of product, m.p. and mixed m.p. with the product from preparation A, 212–213° dec. A second crop was collected; wt. 95 mg., m.p. and mixed m.p. with starting material VIII, 164° dec. (58% recovery of VIII).

6-Dimethylamino-9-(2,3-di-*O*-mesyl-β-D-xylofuranosyl)-2-methylmercaptopurine (VII).—A suspension of 6.1 g. of 6-dimethylamino-9-(2,3-di-*O*-mesyl-5-*O*-trityl-β-D-xylofuranosyl)-2-methylmercaptopurine (VI) in 122 cc. of 80% acetic acid was heated for 25 minutes on the steam-bath, solution being complete in 5 minutes. The solution was evaporated to near dryness *in vacuo*. The sludge was then evaporated *in vacuo* several times with absolute alcohol to remove acetic acid, and the residual crystalline solid was triturated with absolute alcohol and collected; yield 2.3 g. (56%), m.p. 179–181°.

In a pilot run, the yield was 33%, m.p. 181–183°. Recrystallization from absolute alcohol–2-methoxyethanol (methyl Cellosolve) afforded white crystals, m.p. 182–184°; λ_{max}^{KBr} 2.86 μ (OH); 6.24 μ (C=N); 9.05, 9.56 μ (OH and C–O–C); 8.48 μ (sulfonate).

Anal. Calcd. for $C_{18}H_{23}O_8N_5S_3$: C, 36.2; H, 4.65; N, 14.1. Found: C, 36.4; H, 5.17; N, 13.5.

9-(2,3-Anhydro-β-D-ribofuranosyl)-6-dimethylamino-2-methylmercaptopurine (VIII). A. From Dimesylate VII.—A mixture of 1.28 g. of 6-dimethylamino-9-(2,3-di-*O*-mesyl-β-D-xylofuranosyl)-2-methylmercaptopurine (VII), 20 cc. of absolute methanol and 10.3 cc. of 1 *N* methanolic sodium methoxide was refluxed on the steam-bath for 1 hour, solution being complete in 15 minutes. The cooled solution was acidified with 0.69 cc. of acetic acid and evaporated to dryness *in vacuo*. The residue was partially dissolved in 20 cc. of water and extracted with three 15-cc. portions of chloroform. The combined magnesium sulfate-dried extracts were evaporated to dryness *in vacuo* to give 758 mg. (92%) of a sirup. Crystallization from ethyl acetate–heptane gave 285 mg. (34%) of product collected in several crops, m.p. between 164° and 168° dec.

In a pilot run the yield was 100 mg. (25%), m.p. 164–165° dec. Recrystallization from ethyl acetate–heptane gave white crystals, m.p. 168° dec.; λ_{max}^{KBr} 2.99 μ (OH); 6.24 μ (C=N); 9.05, 9.35, 9.45 μ (OH and C–O–C).

Anal. Calcd. for $C_{13}H_{17}O_3N_5S$: C, 48.3; H, 5.30; N, 21.7. Found: C, 48.7; H, 5.74; N, 21.2.

B. From the 2'-*O*-benzoyl-3'-*O*-mesyl Nucleoside XIV.—To a hot solution of 3.75 g. of 9-(2-*O*-benzoyl-3-*O*-mesyl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XIV) in 76 cc. of absolute methanol was added 19.9 cc. of 1 *N* methanolic sodium methoxide, and the solution then was cooled in an ice-bath. Within 1 hour, a solid began to separate. After 18 hours at 0–3°, the mixture was filtered and washed with ice-cold methanol followed by several washings with water to remove sodium methanesulfonate, leaving 1.2 g. (52%) of product, m.p. and mixed m.p. with the material from preparation A, 172–173° dec. Both compounds had identical infrared spectra. Efforts to effect amination of VIII with methanolic or aqueous ammonia at temperatures ranging from 100 to 200° were unsuccessful.

(28) In our experience, periodate oxidations of aminopentofuranosides are always accompanied by overoxidation (unpublished observations of M. J. Weiss, J. P. Joseph, H. M. Kissman, A. M. Small, R. E. Schaub and F. J. McEvoy; see footnote 17 in ref. 10).

(29) Private communication from Miss S. L. Halliday and Dr. J. J. Oleson of these laboratories.

(30) Melting points are uncorrected.

For the tritylation of VIII to the 5'-*O*-trityl-2',3'-anhydronucleoside III see above.

9-(2-*O*-Benzoyl-3,5-*O*-isopropylidene-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (X).—To a solution of 11.2 g. of 6-dimethylamino-9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)-2-methylmercaptopurine (IX)[4] in 112 cc. of reagent pyridine was added 4.3 cc. of benzoyl chloride with ice-cooling. After 18 hours at room temperature in a stoppered flask, the mixture was poured into 550 cc. of iced water and extracted with three 100-cc. portions of methylene chloride. The combined extracts were washed with aqueous sodium bicarbonate, dried with magnesium sulfate and evaporated to dryness *in vacuo* leaving 14.3 g. (100%) of a glass.

Anal. Calcd. for $C_{23}H_{27}O_5N_5S$: C, 57.0; H, 5.60; N, 14.4. Found: C, 57.9; H, 6.24; N, 13.6.

9-(2-*O*-Benzoyl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XI).—A suspension of 14.2 g. of 9-(2-*O*-benzoyl-3,5-*O*-isopropylidene-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (X) in 142 cc. of 70% aqueous acetic acid was stirred at 50° for 2 hours and 15 minutes, solution being complete in 15 minutes. The solution was evaporated to dryness *in vacuo* leaving a sirup. Slow crystallization from toluene gave 6.3 g. (49%) of product, m.p. 184–186°. Recrystallization from absolute alcohol afforded white crystals, m.p. 189–190°, $[\alpha]^{25}D$ −63.7° (1.2% in $CHCl_3$); λ_{max}^{KBr} 2.92 μ (OH); 5.81 μ (ester); 6.26 μ (C=N); 9.05, 9.40, 9.70, 9.90 μ (OH and C–O–C); 13.9 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{20}H_{23}O_5N_5S \cdot H_2O$: C, 51.9; H, 5.40; N, 15.1; H_2O, 3.9. Found: C, 52.6; H, 5.43; N, 15.4; H_2O, 3.4 (Karl Fischer).

9-(2-*O*-Benzoyl-5-*O*-trityl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XII).—A solution of 6.2 g. of 9-(2-*O*-benzoyl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XI) and 4.28 g. of triphenylmethyl chloride in 62 cc. of reagent pyridine was placed in a flask protected with a drying tube and was heated in an oil-bath at 50° for 96 hours. The cooled solution was diluted with 100 cc. of chloroform followed by 300 cc. of water. Solid sodium bicarbonate was added until the mixture was slightly basic. The separated organic phase was dried with magnesium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in toluene and the evaporation repeated leaving 9.6 g. (100%) of product as a glass.

Anal. Calcd. for $C_{39}H_{37}O_5N_5S$: C, 68.1; H, 5.42; N, 10.2. Found: C, 69.0; H, 5.55; N, 9.43.

9-(2-O-Benzoyl-3-*O*-mesyl-5-*O*-trityl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XIII).—To a solution of 6.0 g. of 9-(2-*O*-benzoyl-5-*O*-trityl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XII) in 120 cc. of reagent pyridine, cooled in an ice-bath to 5°, was added 3 cc. of methanesulfonyl chloride at such a rate that the temperature was maintained within the range of 5–9°. The solution then was allowed to stand in a stoppered flask at room temperature for 72 hours. The solution was diluted with 100 cc. of chloroform followed by 500 cc. of iced water. Solid sodium bicarbonate was added until the mixture was slightly basic. The separated organic phase was dried with magnesium sulfate and evaporated to dryness *in vacuo* leaving 6.6 g. (99%) of product as a glass; λ_{max}^{KBr} 5.80 μ (ester); 6.29 μ (C=N); 8.50 μ (sulfonate); 14.2 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{40}H_{39}O_7N_5S_2$: C, 62.8; H, 5.13; N, 9.15; S, 8.36. Found: C, 62.5; H, 5.13; N, 8.24; S, 8.30.

For the conversion of this product to the 5'-*O*-trityl-2',3'-anhydronucleoside III, see above (preparation B).

9-(2-*O*-Benzoyl-3-*O*-mesyl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XIV).—A mixture of 6.35 g. of 9-(2-*O*-benzoyl-3-*O*-mesyl-5-*O*-trityl-β-D-xylofuranosyl)-6-dimethylamino-2-methylmercaptopurine (XIII) and 127 cc. of 80% aqueous acetic acid was heated on the steam-bath for 55 minutes, solution being complete after 5 minutes. The solution was diluted with 300 cc. of water and extracted with three 100-cc. portions of chloroform. The combined extracts were evaporated to dryness *in vacuo* leaving a semi-solid residue. Trituration with several portions of ether to remove triphenylcarbinol afforded 3.8 g. (88%) of product as a glass with λ_{max}^{KBr} 2.96 μ (OH); 5.80 μ (ester); 6.29 μ (C=N); 8.50 μ (sulfonate) and no appreciable absorption at 14.1 μ (monosubstituted phenyl from the triphenylmethyl group). Satisfactory combustion values for this product could not be obtained. For the conversion of this crude product to anhydronucleoside VIII, see above (preparation B).

Methyl 2,5-Di-*O*-Benzoyl-3-phthalimido-3-deoxy-β-D-xylofuranoside (XVI).—A mixture of 5.5 g. of methyl 3-phthalimido-3-deoxy-β-D-xylofuranoside (XV),[10] 55 cc. of reagent pyridine and 5.5 cc. of benzoyl chloride, protected from moisture, was heated on the steam-bath under a condenser for 2.5 hours, solution being complete in 2 minutes. The cooled mixture was diluted with 250 cc. of iced water and extracted with three 100-cc. portions of ethylene dichloride. The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo*. The residue was twice evaporated to dryness *in vacuo* leaving 10.1 g. (107%) of a glass which could not be crystallized, $[\alpha]^{25}D$ +36.7° (2% in $CHCl_3$).

Anal. Calcd. for $C_{28}H_{23}O_8N$: C, 67.0; H, 4.66; N, 2.79. Found: C, 68.5; H, 4.99; N, 2.68.

1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-xylofuranoside (XVII).—To a solution of 10 g. of methyl 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-xylofuranoside (XVI) in 75 cc. of acetic acid and 8.26 cc. of acetic anhydride was added 2.65 cc. of 96% sulfuric acid, dropwise, with ice-bath cooling at such a rate that the temperature was maintained within the range of 10–15°. After 24 hours at room temperature in a stoppered flask, the solution was diluted with 400 cc. of iced water and extracted with three 100-cc. portions of chloroform. The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo*. Crystallization of the sirupy residue gave 7.6 g. (72%) of product, m.p. 118–119°. Recrystallization of a pilot run (56% yield) from absolute alcohol afforded white crystals, m.p. 117–118°, $[\alpha]^{25}D$ +79° (2% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{23}O_9N$: C, 65.9; H, 4.38; N, 2.67. Found: C, 65.8; H, 4.73; N, 2.70.

2,5-Di-*O*-benzoyl-3-phthalimido-3-deoxy-D-xylofuranosyl Chloride (XVIII).—To a solution of 7.6 g. of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-xylofuranoside (XVII) in 7.6 cc. of acetyl chloride was added 200 cc. of anhydrous ether which had been freshly saturated with hydrogen chloride at 0°. A solid separated which was redissolved after 10 minutes of shaking. After 4 days at −3°, protected from moisture, the mixture was evaporated to dryness *in vacuo* (bath temperature 35°). The residue was twice suspended in dry benzene and the evaporation repeated, leaving 7.3 g. (100%) of a glass.

Anal. Calcd. for $C_{27}H_{20}O_7NCl$: C, 64.1; H, 4.00; N, 2.77; Cl, 7.03. Found: C, 64.6; H, 4.34; N, 2.69; Cl, 6.65.

9-(2,5-Di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-xylofuranosyl)-6-chloropurine (XIX).—To a suspension of 5.55 g. of chloromercuri-6-chloropurine[17] in 250 cc. of xylene, which had been dried by distillation of about 50 cc. of xylene, was added a solution of 7.2 g. of 2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-D-xylofuranosyl chloride (XVIII) in 80 cc. of xylene. The mixture was refluxed and stirred for 3 hours while protected from moisture, solution being complete in 30 minutes. The hot solution was filtered through Celite, and the filter cake was washed with three 50-cc. portions of hot chloroform. The combined filtrate and washings were evaporated to dryness *in vacuo*. A solution of the residue in 150 cc. of chloroform was washed with 100 cc. of 30% aqueous potassium iodide and then with water. After drying over magnesium sulfate, the solution was evaporated to dryness *in vacuo*. Crystallization of the residue (8.6 g.) from ethyl acetate–absolute alcohol afforded 4.2 g. (47%) of product in two crops, m.p. 195–196°. Recrystallization from ethyl acetate–absolute alcohol gave white crystals, m.p. 199–201°, $[\alpha]^{25}D$ 0.0° (1.4% in $CHCl_3$); λ_{max}^{KBr} 5.65, 5.84 μ (C=O of phthalimido); 7.95 μ (C=O of benzoyl); 14.0 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{32}H_{22}O_7N_3Cl$: C, 61.6; H, 3.56; N, 11.2; Cl, 5.70. Found: C, 61.7; H, 4.19; N, 11.0; Cl, 5.88.

9-(3-Amino-3-deoxy-β-D-xylofuranosyl)-6-dimethylaminopurine (II).—A solution of 2.83 g. of 9-(2,5-di-*O*-benzoyl-3-phthalimido-3-deoxy-β-D-xylofuranosyl)-6-chloropurine (XIX) in 50 cc. of absolute methanol containing 4.6 cc. of dimethylamine[19] was heated in a steel bomb at 100° for 2

hours. After cooling to room temperature, 6.7 cc. of *n*-butylamine was added and the solution was then refluxed for 18 hours. To the cooled solution 9 g. of wet IRA-400 (OH-form) resin and 25 cc. of water were added and the suspension was stirred for 1 hour. After filtration and washing with methanol, the combined filtrate and washings were evaporated *in vacuo*. The residue (2.1 g.), dissolved in 25 cc. of 50% aqueous methanol, was added to the top of a chromatographic column (2 cm. diam.) packed with 15 g. of wet Amberlite IRC-50 (H-form) resin. The column was washed with 50% aqueous methanol until ultraviolet inspection of the eluates no longer showed purine content (700 cc.). The column was then eluted with 250 cc. of 2 *N* ammonia in 50% aqueous methanol. Ultraviolet inspection of the final 50 cc. of eluate indicated the presence of only a trace amount of nucleoside. Evaporation of the combined fractions to dryness *in vacuo* gave 1.06 g. (80%) of a glass. Slow evaporation of a solution of this glass in ethyl acetate–absolute alcohol gave a crystalline product in 62% yield, m.p. 147–149°, $[\alpha]^{25}D$ −32.7° (2% in H_2O); λ_{max}^{KBr} 3.0 μ (OH and NH_2); 6.24 μ (C=N); 9.04, 9.46, 9.70 μ (C–OH and C–O–C); $\lambda_{max}^{alc.}$ 274 mμ (ϵ 18,800).

Anal. Calcd. for $C_{12}H_{18}O_3N_6$: C, 49.0; H, 6.17; N, 28.6. Found: C, 49.4; H, 6.57; N, 28.3.

This compound consumed 1.0 mole-equivalents of periodate in aqueous sodium bicarbonate in 17 minutes, and an additional 0.6 equivalent in 6 days.

6-Dimethylamino-9-(3-vanillylideneamino-3-deoxy-β-D-xylofuranosyl)-purine.—A solution of 100 mg. of crystalline 9-(3-amino-3-deoxy-β-D-xylofuranosyl)-6-dimethylaminopurine (II) and 80 mg. of vanillin in 20 cc. of 75% aqueous alcohol was refluxed on the steam-bath for 1 hour. Evaporation of this solution *in vacuo* produced a glass, crystallization of which from ethyl acetate gave 140 mg. (96%) of product, m.p. 194–196°. In another run, crude II gave 460 mg. (83%) of product, m.p. 192–194°. Recrystallization from aqueous ethanol of the product obtained from a pilot run (77% yield) gave white crystals, m.p. 195–196°, $[\alpha]^{25}D$ +90° (0.7% in MeOH).

Anal. Calcd. for $C_{20}H_{24}O_5N_6$: C, 56.1; H, 5.65; N, 19.6. Found: C, 56.2; H, 5.95; N, 19.4.

3-Amino-3-deoxy-D-xylose Hydrochloride.—A solution of 200 mg. of crystalline 9-(3-amino-3-deoxy-β-D-xylofuranosyl)-6-dimethylaminopurine (II) in 5 cc. of 1% hydrochloric acid, to which 0.114 cc. of concentrated hydrochloric acid had been added, was refluxed for 3 hours. To the partially cooled solution was added a hot solution of 172 mg. of picric acid in 2 cc. of absolute alcohol. Yellow crystals of 6-dimethylaminopurine picrate immediately separated. The picrate was collected and washed with water and alcohol; yield 210 mg. (79%), m.p. 247°, mixed melting point with an authentic sample showed no depression.

The filtrate, obtained after removal of 6-dimethylaminopurine picrate, was washed with six 10-cc. portions of chloroform to remove picric acid, and was then decolorized with Norit and evaporated to a sirup *in vacuo* (bath temperature 40–50°). Trituration with acetic acid, to which had been added a few drops of water, afforded crystals; yield 83 mg. (66%), m.p. 167° dec. Recrystallization from 0.5 cc. of water by addition of 6 cc. of acetic acid gave white crystals, m.p. 169° dec. The compound gave an infrared spectrum identical with that of authentic 3-amino-3-deoxy-D-xylose hydrochloride.[10]

Anal. Calcd. for $C_5H_{11}O_4N\cdot HCl$: C, 32.4; H, 6.52; N, 7.55. Found: C, 32.4; H, 7.14; N, 7.33.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF ALABAMA MEDICAL CENTER]

Reactions of Carbohydrates with Nitrogenous Substances. VI. The Amadori Rearrangement in Methanol[1,2]

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The rearrangement of N-*p*-tolyl-D-glucosylamine (GPT) to 1-deoxy-1-*p*-toluino-D-fructose (DTF), an example of the Amadori rearrangement, appears to be subject to generalized acid–base catalysis. The reaction has been studied in methanol solution at 100° by use of a series of carboxylic acids and phenols and their salts. Yields as high as 70% were obtained. The production of a high yield is dependent upon minimizing at least three destructive reactions. These are: (1) an acid-catalyzed destruction of the product (DTF) not accompanied by much color formation; (2) a base-catalyzed destruction of DTF accompanied by considerable color formation; and (3) an acid-catalyzed destruction of GPT with much color formation.

Introduction

The rearrangement of N-*p*-tolyl-D-glucosylamine (GPT) to 1-deoxy-1-*p*-toluino-D-fructose (DTF) is an example of the Amadori rearrangement.[3] Amadori[4] was able to obtain either GPT or DTF depending upon the experimental conditions and thought that both isomers were D-glucose derivatives. Kuhn and Weygand[5] demonstrated that one of the isomers was actually a D-fructose derivative (DTF).

Weygand[6] later established a general method for the preparation of N-substituted 1-amino-1-deoxy-D-fructose compounds by fusion of D-glucose with an amine on a boiling water-bath in the presence of a small amount of aqueous hydrochloric or acetic acid. This method was successful only with primary arylamines; hence the Amadori rearrangement was long thought to be limited to aldosyl derivatives of primary arylamines.[7] The rearrangement of D-xylosylpiperidine to 1-deoxy-1-piperidino-D-xylulose, obtained as the 5-trityl derivative, during tritylation[8] was apparently overlooked.

Recent findings have shown the rearrangement

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(2) Previous paper, this series: L. Rosen, J. W. Woods and W. Pigman, *J. Org. Chem.*, **22**, 1727 (1957).

(3) For a review which includes many references, see J. E. Hodge, *Adv. Carbohydrate Chem.*, **10**, 169 (1955).

(4) M. Amadori, *Atti accad. Lincei*, [6] **13**, 72 (1931); *C. A.*, **25**, 5147 (1931). In an earlier paper (*ibid.*, [6] **9**, 68 (1929)), Amadori discussed the problem of the isomers arising from the condensation of D-glucose and *p*-phenetidine.

(5) R. Kuhn and F. Weygand, *Ber.*, **70**, 769 (1937).

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(7) L. I. Smith and R. H. Anderson, *J. Org. Chem.*, **16**, 963 (1951).

(8) K. Zeile and W. Kruckenberg, *Ber.*, **75**, 1127 (1942).