Ribosyl Derivatives of Hypoxanthine[1]

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The preparation of 7-α- and 7-β-D-ribofuranosylhypoxanthines (α- and β-**5c**) from 9-propenylhypoxanthine (**10**) is described. β-**5c** was also prepared from the chloromercuri derivative of 3-benzylhypoxanthine (**1**), but the chloromercuri derivative of 3-benzhydrylhpyoxanthine (**2**) gave, after removal of the protective groups, not 7- but 1-β-D-ribofuranosylhypoxanthine (β-**13c**) resulting presumably from a rearrangement of the benzhydryl group to N-9. β-**13c** was also prepared from 3-benzhydrylhypoxanthine (**3**) and the mercuri derivative of 9-propenylhypoxanthine. The 7 isomer of 6-mercaptopurine ribonucleoside was prepared from β-**5a**.

We have been engaged in the synthesis of the nucleoside components of the family of B_{12} vitamins, and one of the objectives of the present work was to prepare the nucleoside component of factor *G*, 7-α-D-ribofuranosylhypoxanthine, and congeners thereof.[1] Previously we found that the chloromercuri derivative of 3-benzylhypoxanthine (**1**) reacts with acylglycosyl halides at N-7 to give, for example, 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-benzylhypoxanthine (β-**4b**), which, on treatment with sodium methoxide in methanol, gave 3-benzyl-7-β-D-ribofuranosylhypoxanthine (β-**4c**).[2,3] Catalytic hydrogenolysis of β-**4c** gave some of the desired 7-β-D-ribofuranosylhypoxanthine (β-**5c**), but the major product was 3-benzyl-1,2-dihydro-7-β-D-ribofuranosylhypoxanthine.[2,3] In the present work this procedure was improved by reversing the latter two steps and using benzoyl blocking groups: thus β-**4a** was hydrogenolyzed to β-**5a**, which was then debenzoylated to β-**5c**. The benzoylated nucleoside β-**5a** was also thiated to give 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine-6(1*H*)-thione (β-**6a**), which was debenzoylated to 7-β-D-ribofuranosylpurine-6(1*H*)-thione (β-**6c**), an isomer of the anticancer agent 6-mercaptopurine ribonucleoside.

Even though the route β-**4a** to β-**5a** to β-**5c** is an improvement over the original procedure,[3] a large amount of the unwanted 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3-benzyl-1,2-dihydrohypoxanthine is still obtained. In an effort to improve the yield of β-**5c** by reducing the amount of dihydro compound formed, the more readily hydrogenolyzed benzhydryl group was substituted for the benzyl group of **1**. Contrary to the previous report,[3] however, the reaction of chloromercuri 3-benzhydrylhypoxanthine (**2**) with 2,3,5-tri-*O*-acetylribofuranosyl chloride did not give 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-benzhydrylhypoxanthine (β-**4b**). Removal of the blocking groups of the product gave what appeared to be a 1-ribosylhypoxanthine (**13c**). Furthermore, no ring-reduced material was formed. Thus, it would seem that **2** rearranged to the mercuri derivative of 9-benzhydrylhypoxanthine (**8**),[4] which reacted with the halo sugar at N-1 to give 9-benzhydryl-1-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)hypoxanthine (**14b**)[5] (Scheme I).

Since 3-benzylhypoxanthine is known to be alkylated at N-1 in dipolar aprotic solvents,[6,7] 3-benzhydrylhypoxanthine (**3**) was allowed to react with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in N,N-dimethylacetamide (DMA). From this reaction was isolated 1-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)-3-benzhydrylhypoxanthinium bromide (**7b**), identified by its elemental analyses and spectra. Initial attempts to purify **7b** by recrystallization from boiling ethanol resulted, in part, in loss[7] of the labile 3-benzhydryl group to give **13b** and, in part, in migration of this group from N-3 to N-9[6,7] to give **14b**. Removal of the acetyl groups of **13b** gave **13c**, identical with that obtained from **2**. In both cases only one anomer was obtained, and its anomeric configuration was established as β in two ways. First, **13c** was converted into its 2′,3′-isopropylidene derivative **12**, the pmr spectrum of which showed a coupling constant of 2.2 Hz from the $H_{1'}$–$H_{2'}$ coupling, precluding the α, or *cis*, configuration.[8] Second, 1-β-D-ribofuranosylhypoxanthine (β-**13c**) was also synthesized from 9-propenylhypoxanthine[9] (**10**) *via* its mercuri derivative **9**.[10] 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-9-propenylhypoxanthine (β-**15b**) was deacetylated, and the propenyl group of β-**15c** was removed by oxidation in neutral solution[9] to give β-**13c**. Although this method of synthesis of β-**13c** is definitely inferior to its preparation from **3**, it does constitute a proof of structure of β-**13c** based on the *trans* rule.[11]

In still another approach to the synthesis of 7-ribosylhypoxanthine (**5c**), 9-propenylhypoxanthine (**10**) was allowed to react with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in N,N-dimethylacetamide at room temperature. Under these conditions 7-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)-9-propenylhypoxanthinium bromide (**11a**) was formed.[7,12] Oxidative removal of the propenyl group of **11a** gave 7-(2,3,5-tri-*O*-benzoyl-D-ribofuranosyl)hypoxanthine (**5a**), which was purified by chromatography on a silica gel column before debenzoylation to 7-D-ribofuranosylhypoxanthine (**5c**). Examination of the nmr spectrum of **5c** indicated that it was an approximately 1:1 mixture of the α and β anomers.[13] The formation of equal quantities of α and β anomers of

(1) This work was supported by funds from the Southern Research Institute, the C. F. Kettering Foundation, and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51. Preliminary communications describing part of this work have appeared: J. A. Montgomery and H. J. Thomas, *J. Heterocycl. Chem.*, **5**, 303, 741 (1968).

(2) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **28**, 2304 (1963).

(3) H. J. Thomas and J. A. Montgomery, *ibid.*, **31**, 1413 (1966).

(4) The exact nature of this proposed intermediate (**8**) is obviously not known.

(5) The migration of the benzyl group from the 3 to the 9 position of hypoxanthines is known.[6,7]

(6) J. A. Montgomery and H. J. Thomas, *Chem. Ind.* (London), 1596 (1965).

(7) J. A. Montgomery, K. Hewson, S. J. Clayton, and H. J. Thomas, *J. Org. Chem.*, **31**, 2202 (1966).

(8) N. J. Leonard and R. A. Laursen, *J. Amer. Chem. Soc.*, **85**, 2026 (1963).

(9) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **30**, 3235 (1965).

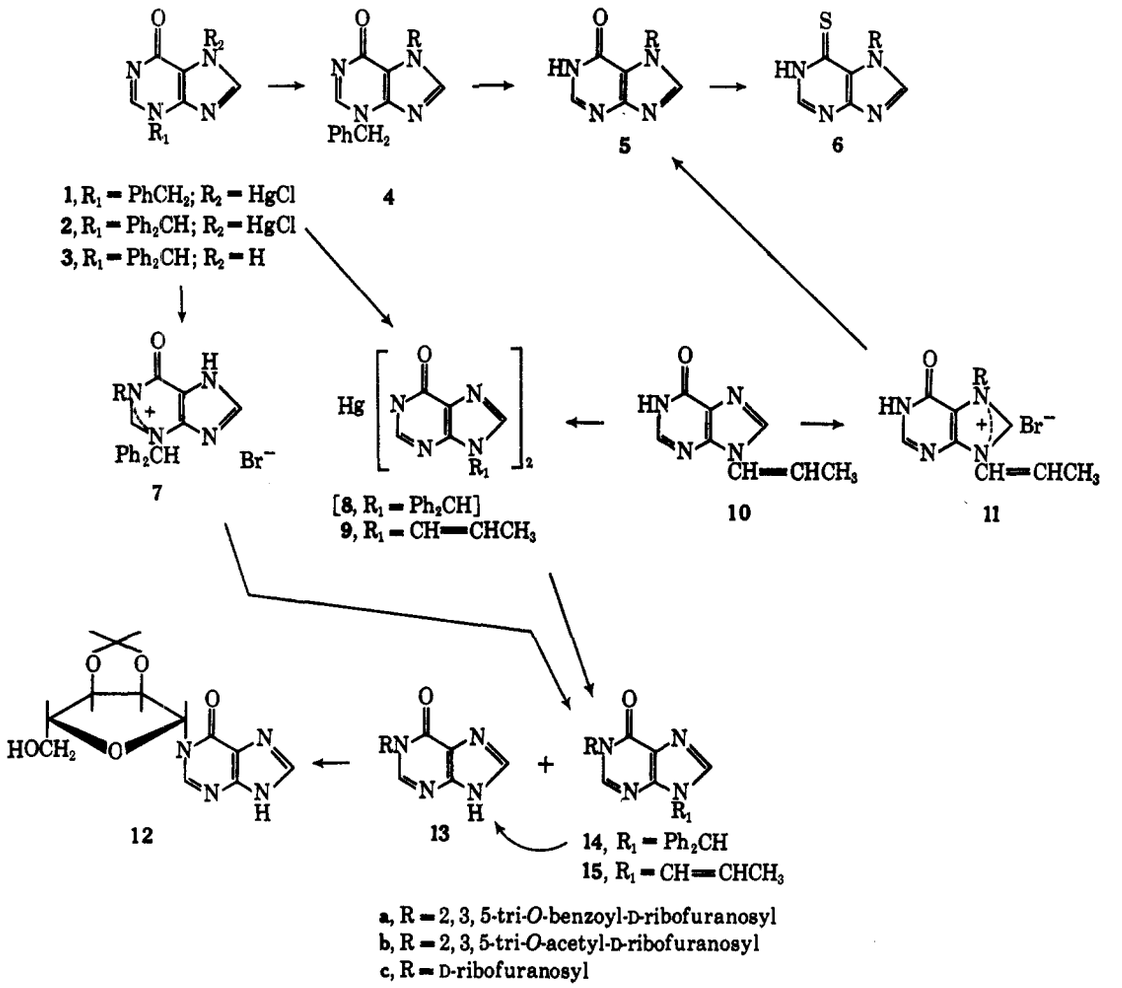
(10) See also T. H. Hashizume and H. Yamazaki, *Tetrahedron Lett.*, 3839 (1967).

(11) B. R. Baker, *Ciba Found. Symp. Chem. Biol. Purines*, 120 (1957).

(12) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, **84**, 1914 (1962).

(13) J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **11**, 48 (1968).

SCHEME I



a glycosylpurine from the reaction of a purine with a glycosyl halide containing a participating acyloxy group at C-2 has not been previously reported. The amount of α anomer obtained from **10** could be increased by allowing it to react with 5-*O*-benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate,[14-17] which gave an anomer ratio of about 2α to 1β. Separation of these anomers was achieved by chromatography on a cellulose column. The β anomer was identical with that prepared from **1**. The identity of the *cis* or α anomer was established by analysis and by its ultraviolet and pmr spectra. The signal due to $H_{1'}$ of α-**5c** (*cis* anomer) occurs downfield from the signal due to $H_{1'}$ of β-**5c** (*trans* anomer), as is the case in all reported instances of anomeric purine nucleoside pairs.[13,15,18,19] Yet another exception to Hudson's rules, however, is presented by this anomeric pair (α- and β-**5c**). It is probably significant that the 7-D-ribofuranosyladenines obey Hudson's rules, whereas the 7-D-ribofuranosylguanines[19] and hypoxanthines, both of which have an oxo function at C-6, do not.

In an effort to better understand the reaction of 9-propenylhypoxanthine (**10**) with these glycosyl halides, their anomeric configurations were determined by means of nmr spectrometry. 2,3,5-Tri-*O*-acetylribofuranosyl bromide (and also the chloride) was shown to be a 3:2 mixture of β to α anomer, the 5-benzoylribofuranosyl bromide 2,3-cyclic carbonate was found to be 2:1 β to α mixture, but the 2,3,5-tri-*O*-benzoylribofuranosyl bromide was a 1:2 β to α mixture.[20,21] Based on these analyses the anomeric mixtures of nucleosides formed could be explained by postulating an SN2-type reaction. The reaction of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide[22] with **10**, however, gave, after removal of the protective groups, 7-α-D-arabinofuranosylhypoxanthine,[3] with at most a trace of the β anomer. This result requires the complete anomerization of the α-bromide, which is highly unlikely, or the intervention of the 1,2 ortho ester ion. Why ortho ester ion interven-

(14) R. S. Wright, G. M. Tener, and H. G. Khorana, *J. Amer. Chem. Soc.*, **80**, 2004 (1958).

(15) J. A. Montgomery and H. J. Thomas, *ibid.*, **87**, 5442 (1965).

(16) Reaction of this glycosyl halide with the mercuri derivative of *N*-benzoyl-3-benzyladenine gave an anomeric mixture which after removal of the blocking groups gave a 14% yield of 7-α-D-ribofuranosyladenine and an 8% yield of the β anomer.[15] Wright, *et al.*,[14] similarly obtained a 24% yield of 9-α-D-ribofuranosyladenine and a 15% yield of adenosine. This halo sugar probably reacts by direct displacement of the bromo group with Walden inversion, and evidence has been presented to support the contention that the sugar is an anomeric mixture with the β anomer predominating[17] (prior to pmr work reported herein).

(17) G. M. Tener and H. G. Khorana, *J. Amer. Chem. Soc.*, **79**, 437 (1957).

(18) T. Nishimura and B. Shimuzu, *Chem. Pharm. Bull.* (Tokyo), **13**, 803 (1965).

(19) K. Imai, A. Nohara, and M. Honjo, *ibid.*, **14**, 1378 (1966).

(20) Other investigators have also reported that this sugar is richer in the α anomer.[21]

(21) J. D. Stevens, R. K. Ness, and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1806 (1968).

(22) R. K. Ness and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **80**, 2007 (1958).

tion occurs with the *arabino* and apparently does not with the *ribo* sugar is not easily explained.

Stevens, Ness, and Fletcher have observed that the reaction of 2,3,5-tri-*O*-benzoylribofuranosyl bromide with 5,6-dimethylbenzimidazole in dioxane gave a 1.8 β to 1 α anomer ratio.[21] In the related Hilbert–Johnson reaction of 5-benzoyloxymethyl-2,4-dimethoxypyrimidine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride in *acetonitrile*, a *polar solvent*, both α and β anomers are formed also,[23] although with other sugars and other pyrimidines only the β anomer is produced (in the *nonpolar solvents benzene, toluene, and xylene*, a number of instances have been reported in which both anomers are formed).[24] Prystas and Sorm have suggested that the stronger nucleophiles (among the 2,4-dialkoxypyrimidines) can react with the *trans* halogenoses by direct displacement, thus successfully competing with ortho ester ion formation, which would give rise to *trans* (or in this case β) nucleosides only.[25] This explanation obviously cannot, without modification, be applied to the present case.

Experimental Section

The melting points reported were determined with a Mel-Temp apparatus and are not corrected. The optical rotations were determined in the solvents specified with a Rudolph Model 80 polarimeter. The uv spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer (Table I). The ir spectra of the compounds were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer, but the data are not presented. The nmr spectra were determined in DMSO-d_6 containing TMS as internal reference with a Varian A-60A spectrometer. Chromatographic analyses were carried out on thin layer plates of silica gel H (Brinkmann). The plates were developed using mixtures of $CHCl_3$ and MeOH in various proportions. The spots were detected by uv light after spraying the plates with Ultraphor (WT, highly concentrated) (BASF Colors & Chemicals, Inc., Charlotte, N. C.). Most of the chromatographic purifications were carried out on Mallinckordt SilicAR-7 with the solvents indicated; exceptions are noted. The analytical samples were dried over P_2O_5 at 0.07 mm for 16–20 hr at the temperatures given.

7-α- and 7-β-D-Ribofuranosylhypoxanthine (α- and β-5c). A.—A solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide, prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (8.43 g, 16.7 mmol) and 9-propenylhypoxanthine (2.68 g, 15.2 mmol) in DMA (200 ml) was left for 4.5 days at room temperature and then evaporated to dryness *in vacuo*. The gummy residue became a solid upon trituration with Et_2O. A solution of the solid in MeOH (500 ml) was stirred and cooled in an ice bath while 4% aqueous $KMnO_4$ (100 ml) was added slowly. The resulting brown solid was filtered off and washed with MeOH. The combined filtrate and washings were evaporated to dryness *in vacuo*, and the residue was triturated with $CHCl_3$ (200 ml). The $CHCl_3$-insoluble material was removed by filtration, and the filtrate was dried over $MgSO_4$ and evaporated to dryness. The residue obtained was a white glass weighing 5.14 g. Purification was effected by column chromatography (4 cm × 20 cm) with $CHCl_3$–MeOH (97:3) as the eluent. The product, a mixture of 7-α- and 7-β-D-(2,3,5-tri-*O*-benzoyl)ribofuranosylhypoxanthines, was obtained as a white glass weighing 3.12 g.

A solution of the blocked nucleosides in MeOH (61 ml) containing NaOMe (581 mg, 10.8 mmol) was refluxed for 45 min, chilled in an ice bath, stirred with Amberlite IR-120 (H) ion-exchange resin to remove Na^+ ions, and then evaporated to dryness *in vacuo*. An aqueous solution of the residue was washed with $CHCl_3$ to remove methyl benzoate, and, after treatment with charcoal, was evaporated to dryness. The residue, a mixture of 7-α- and 7-β-D-ribofuranosylhypoxanthines, became a white solid after trituration with EtOH: yield 1.04 g (35%). This solid was found by nmr spectrometry to be a 1:1 mixture of α and β anomers.

The anomers were separated by chromatographing 953 mg of the mixture on an Avicel[26] column (3.5 cm × 45 cm) using H_2O as the eluent and collecting fractions of 5 ml. The fractions were examined by tlc on Avicel plates developed in H_2O; fractions rich in the faster moving α anomer were combined and evaporated to dryness. The residual white solid weighed 470 mg. There was also obtained, by combining the other fractions, 376 mg of a 1:1 mixture and 92 mg of a mixture rich in β anomer.

The mixture rich in α anomer was rechromatographed on an Avicel column, and 127 mg of nearly pure α anomer was obtained. Pure α-5c was precipitated as a gel, first from H_2O, and then from aqueous EtOH. It was dried at 100°: yield 35 mg; $[\alpha]^{25}D$ −78.1 ± 0.3 (*c* 0.99, H_2O); δ (ppm) 3.57 (m, C_5'-H_2), 4.20 (m, C_2'-H, C_3'-H, and C_4'-H), 5.37 (m, OH, NH), 6.66 (d, $J_{1'2'}$ = 4.4 Hz, C_1'-H), 8.00 and 8.39 (C_2-H and C_8-H).

Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 44.79; H, 4.51; N, 20.90. Found: C, 45.04; H, 4.67; N, 20.98.

B.—A solution of 5-*O*-benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate, prepared from methyl 5-*O*-benzoyl-β-D-ribofuranoside 2,3-cyclic carbonate (3.23 g, 11.0 mmol), and 9-propenylhypoxanthine (**10**, 1.76 g, 10.0 mmol) in DMA (100 ml) was left at room temperature for 4 days and then evaporated to dryness *in vacuo*. Trituration of the residue with Et_2O gave a buff-colored solid. A solution of this solid in MeOH (500 ml) was stirred and chilled in an ice bath while 4% aqueous $KMnO_4$ (100 ml) was slowly added. The resulting brown precipitate was removed by filtration and washed with MeOH. The combined filtrate and washings were evaporated to dryness *in vacuo*, the residue was triturated with boiling $CHCl_3$ (300 ml), and the insoluble material was removed by filtration. The $CHCl_3$ filtrate was dried over $MgSO_4$ and evaporated to dryness. The colorless glass (1.1 g) obtained was purified by column chromatography (4 cm × 25 cm) eluting first with $CHCl_3$ (1650 ml) and then with 95:5 $CHCl_3$–MeOH (600 ml) to give a glass weighing 100 mg. This material was dissolved in MeOH (26 ml) containing NaOMe (54 mg, 1.0 mmol), and the solution was refluxed for 30 min, neutralized with glacial AcOH, and evaporated to dryness *in vacuo*. An aqueous solution of the residue was extracted with $CHCl_3$, which on evaporation to dryness gave a white solid.

The $CHCl_3$-insoluble material was extracted with MeOH, and the inorganic solid was removed by filtration. Evaporation of the filtrate gave a white glass (2.01 g). Cleavage of the cyclic carbonate was effected with MeOH (78 ml) containing NaOMe (162 mg, 3.0 mmol). A white solid was isolated as described above.

The combined solids from the two methoxide treatments were purified by column chromatography (2.5 cm × 40 cm); $CHCl_3$–MeOH (3:1) was the eluate. The product was obtained as a white solid (1.05 g), which was further purified by chromatography on two Avicel plates. The product was obtained as a white solid upon eluting the combined α and β bands with H_2O: yield 950 mg. This material was found by uv spectrophotometry to be 65% **5c**, thus giving a yield of 23%. The nmr spectrum of this solid showed that it was a 2:1 mixture of α- and β-5c.

7-β-D-Ribofuranosylhypoxanthine (β-5c).—A solution of 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-3-benzylhypoxanthine[3] (β-**4a**, 1.34 g, 2.00 mmol) in EtOH (200 ml) was hydrogenolyzed with 50 psi of H_2 in the presence of 5% Pd–C (400 mg) at 80° and for 18 hr. The catalyst was removed by filtration and washed with EtOH. The filtrate and washings were combined and evaporated to dryness *in vacuo*. A white glass weighing 1.22 g was obtained. Examination of this material by tlc showed that it was a mixture of 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-hypoxanthine (β-**5a**) and 3-benzyl-2,3-dihydro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)hypoxanthine.[3] Separation of the two products was effected by column chromatography using $CHCl_3$–MeOH (97:3) as the eluent. The ring-reduced compound was obtained first in a total yield of 662 mg (50%). The desired product (β-**5a**) was then obtained in a total yield of 393 mg (34%).

A solution of the blocked nucleoside β-**5a** in MeOH (21.3 ml) containing NaOMe (72.4 mg, 1.34 mmol) was refluxed for 0.5 hr, neutralized with AcOH, and evaporated to dryness *in vacuo*.

(23) M. Prystas and F. Sorm, *Collect. Czech. Chem. Commun.*, **31**, 1053 (1966).

(24) A comprehensive review of the Hilbert–Johnston reaction including its stereochemistry has recently appeared: J. Pliml and M. Prystas, *Advan. Heterocycl. Chem.*, **8**, 115 (1967).

(25) M. Prystas and F. Sorm, *Collect. Czech. Chem. Commun.*, **31**, 1035 (1966).

(26) American Viscose Division of FMC Corp., Newark, Del.

The residue was dissolved in H_2O (20 ml). The solution was washed with $CHCl_3$ (two 20-ml portions) and evaporated to 4 ml, and the precipitate that formed was collected by filtration: yield 74 mg (14% over-all yield).

The analytical sample of β-**5c** was obtained by recrystallization from MeOH. It was dried at 78°: mp 216–218°; $[\alpha]^{25}D$ +30.0 ± 1.3 (*c* 0.44, H_2O); δ (ppm) 3.68 (m, C_5'-H_2), 4.00 and 4.38 (m, C_2'-H, C_3'-H, and C_4'-H), 6.23 (d, $J_{1'2'}$ = 4.3 Hz, C_1'-H), 8.03 and 8.60 (C_2-H and C_8-H).

Anal. Calcd for $C_{10}H_{12}N_4O_5$: C, 44.79; H, 4.51; N, 20.90. Found: C, 44.75; H, 4.56; N, 20.65.

7-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)purine-6(1*H*)-thione (β-**6a**).—To a solution of 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)hypoxanthine (β-**5a**, 1.46 g, 2.52 mmol) in pyridine (40 ml) was added P_2S_5 (2.47 g, 11.1 mmol). The resulting suspension was stirred and refluxed for 10 min to give a clear solution. Addition of 2 drops of H_2O created the desired orange turbidity, and reflux was continued for 4 hr. The orange, turbid mixture was chilled, and the liquid portion was decanted. The liquid was evaporated to a thin syrup. Both the orange solid and the thin syrup were added portionwise to 1 l. of boiling H_2O. Boiling was continued for 0.5 hr. The mixture was cooled, and the solid was collected by filtration. A $CHCl_3$ solution of the solid was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The yellow glass (1.35 g) obtained was purified by column chromatography (3.2 cm × 22 cm); EtOAc was the eluent. The product (β-**6a**) crystallized from C_6H_6–EtOAc: yield 612 mg (41%) [366 mg (25%) of β-**5a** was recovered].

An analytical sample of β-**6a** was obtained by recrystallization from C_6H_6–EtOAc. The sample was dried at 100°: λ_{max}, nm (ε × 10^{-3}), 0.1 *N* HCl 243 (34.9), 345 (17.4); pH 7 235 (39.2), 342 (15.0); 0.1 *N* NaOH 226 (34.9), 319 (15.0).

Anal. Calcd for $C_{31}H_{24}N_4O_7S$: C, 62.41; H, 4.05; N, 9.39. Found: C, 62.35; H, 4.06; N, 9.33.

7-β-D-Ribofuranosylpurine-6(1*H*)-thione (β-**6c**).—A solution of 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)purine-6(1*H*)-thione (β-**6a**, 495 mg, 0.85 mmol) in dry MeOH (52 ml) containing NaOMe (108 mg, 2.0 mmol) was refluxed for 45 min, chilled in an ice bath, and treated with Amberlite IR-120 (H) ion-exchange resin to remove Na^+ ions. The resin was filtered off and washed with H_2O. The combined filtrate and washings were evaporated to dryness *in vacuo*. An aqueous solution of the residue was washed with $CHCl_3$. Concentration of the aqueous solution to 10 ml gave 14 mg of crystalline purine-6(1H)-thione that was collected by filtration. The gel that formed in the filtrate was collected by filtration. The solid weighed 91 mg and was found by tlc to be β-**6c** contaminated with purine-6(1*H*)-thione. The product was purified by preparative tlc; $CHCl_3$–MeOH (3:1) was the developing solvent. The product band was eluted with boiling MeOH. Evaporation of the MeOH gave a white solid, which was dried at 100°: yield 45 mg (19%); λ_{max}, nm (ε × 10^{-3}), 0.1 *N* HCl 221 (8.80), 331 (15.7); pH 7 221 (8.80), 328 (15.5); 0.1 *N* NaOH 230 (10.6), 318 (15.3).

Anal. Calcd for $C_{10}H_{12}N_4O_4S$: C, 42.25; H, 4.25; N, 19.71. Found: C, 42.20; H, 4.15; N, 19.78.

3-Benzhydryl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)hypoxanthinium Bromide (β-**7b**).—A solution of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide, prepared from 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose (4.77 g, 15.0 mmol), and 3-benzhydrylhypoxanthine (**3**, 3.02 g, 10.0 mmol) in DMA (105 ml) was kept at room temperature for 5 days and then evaporated to dryness *in vacuo*. Trituration of the residue with two 200-ml portions of Et_2O followed by two 200-ml portions of $CHCl_3$ produced a white powder: yield 2.51 g (39%).

The analytical sample was obtained by filtering a solution of the solid (100 mg) in 200 ml of 50% EtOH–$CHCl_3$ and then evaporation of the filtrate without heat. The white residue was triturated with $CHCl_3$ and collected by filtration before it was dried at room temperature: yield 88 mg; δ (ppm) 1.97, 2.03, and 2.13 (CH_3 of acetyl), 4.07 and 4.37 (m, C_5'-H_2, C_4'-H, and H_2O), 5.35 (C_4'-H), 5.77 (C_3'-H), 6.17 (d, $J_{1'2'}$-*ca.* 2 Hz, C_1'H), 7.44, 7.61 (Ph_2CH), 8.55 (C_2-H), 9.00 (C_8-H).

Anal. Calcd for $C_{29}H_{29}BrN_4O_8 \cdot 1.5H_2O$: C, 52.10; H, 4.82; N, 8.39. Found: C, 52.24; H, 4.61; N, 8.47.

The Mercuri Salt of 9-Propenylhypoxanthine (**9**).—To a solution of $HgCl_2$ (2.72 g, 10.0 mmol) in EtOH (200 ml) was added 9-propenylhypoxanthine (**10**, 3.52 g, 20.0 mmol). The resulting suspension was stirred while 1 *N* NaOH (20 ml) was slowly added. The resulting yellow color was quickly dispelled by heating the reaction mixture to the boiling point, and the white suspension was diluted with 400 ml of H_2O and chilled. The white solid was collected by filtration, washed with H_2O until free of Cl^- ions, and dried for 2.5 hr at 100° (0.07 mm) over P_2O_5: yield 5.14 g (90%).

The analytical sample was obtained by dissolving some of the compound in hot EtOH, filtering the solution, and evaporating it to dryness. The residue was dried at 100°.

Anal. Calcd for $C_{16}H_{14}HgN_8O_2 \cdot 1H_2O$: C, 33.77; H, 2.83; N, 19.69. Found: C, 33.90; H, 2.67; N, 19.13.

1-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)hypoxanthine (**12**).—To 50 ml of Me_2CO was added 2,2-dimethoxypropane (0.17 ml) followed by 70% $HClO_4$ (0.22 ml). The resulting solution was stirred at room temperature for 5 min before adding 1-β-D-ribofuranosylhypoxanthine (β-**13c**, 128 mg, 0.48 mmol). The solid dissolved in 15 min, and stirring was continued for 20 min longer. The solution was neutralized with pyridine (0.25 ml) and evaporated to dryness *in vacuo*. To the residue suspended in H_2O (30 ml) was added concentrated NH_4OH (three 25-portions). The resulting solution was extracted with CH_2Cl_2 (two 30-ml portions) and then evaporated to 20 ml, whereupon a precipitate formed. The precipitate was collected by filtration: yield 99 mg (67%).

The analytical sample of **12** was obtained by recrystallization from H_2O. It was dried at 100°: mp 271–273°; λ_{max}, nm (ε × 10^{-3}), 0.1 *N* HCl 248 (9.44); pH 7 251 (8.90); 0.1 *N* NaOH 261 (9.60); δ (ppm) 1.30 and 1.52 (CH_3), 3.66 (d, C_5'-H), 4.19 (m, C_4'-H), 4.98 (m, C_2'-H and C_3'-H), 6.22 (d, $J_{1'2'}$ = 2.2 Hz, C_1'-H), 8.17 (C_8-H), 8.47 (C_2-H).

Anal. Calcd for $C_{13}H_{16}N_4O_5$: C, 50.65; H, 5.23; N, 18.17. Found: C, 50.59; H, 5.35; N, 18.37.

1-β-D-Ribofuranosylhypoxanthine (β-**13c**).[3] **A.**—A solution of 3-benzhydryl-1-β-D-(2,3,5-tri-*O*-acetyl)ribofuranosylhypoxanthinium bromide (β-**7b**, 1.62 g, 2.43 mmol) in 1 l. of EtOH was refluxed for 30 min and evaporated to dryness. A solution of the residue in $CHCl_3$ was dried over $MgSO_4$ and evaporated to dryness. A solution of this residue in dry MeOH (54 ml) containing NaOMe (222 mg, 4.12 mmol) was refluxed for 30 min, then stirred with Amberlite IR-120 (H) ion-exchange resin, and evaporated to dryness *in vacuo*. The residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ solution was dried over $MgSO_4$ and evaporated to dryness. A gelatinous like residue was obtained that weighed 400 mg (38%). Spectral data indicate that this material is 9-benzhydryl-1-β-D-ribofuranosylhypoxanthine (β-**14c**). The analytical sample was obtained by precipitation from $CHCl_3$–petroleum ether. It was dried at 78°: δ (ppm) 3.67 (C_5'-H_2), 4.00 (m, C_4'-H, C_3'-H, and C_2'-H), 5 (broad m, OH), 6.15 (d, $J_{1'2'}$ = *ca* 4 Hz, C_1'-H), 7.08, 7.35 (Ph_2CH), 7.96 (C_8-H), and 8.68 (C_2-H).

Anal. Calcd for $C_{23}H_{22}N_4O_5$: C, 63.59; H, 5.10; N, 12.90 Found: C, 63.64; H, 4.91; N, 12.75.

The H_2O solution was evaporated to dryness giving a white glass that was chromatographically homogeneous. It crystallized from a small amount of H_2O on seeding: yield 200 mg of β-**13c**; δ (ppm) 3.68 (m, C_5'-H_2), 4.00 (m, C_2'-H, C_3'-H, and C_4'-H), 5.50 (broad m, OH and NH), 6.13 (d, $J_{1'2'}$ = 4.2 Hz, C_1-H), 8.09 (C_8-H), 8.55 (C_2-H). This material is identical with that prepared from **2**.[3]

Evaporation of the aqueous filtrate to dryness produced a glass weighing 288 mg. This material was found by uv spectrophotometry to be 45% β-**13c**. The total yield of β-**13c** was 51%.

B.—To a cold solution of 9-propenyl-1-β-D-ribofuranosylhypoxanthine (β-**15c**, 368 mg, 1.20 mmol) in 0.5 *N* methanolic NaOH (20 ml) and H_2O (2 ml) was added dropwise 4% $KMnO_4$ solution (12 ml). The resulting brown precipitate was filtered off and washed with H_2O. The filtrate and washings were combined and stirred with Amberlite IR-120 (H) ion-exchange resin until pH 5 was attained. The resin was filtered off and washed with H_2O. The filtrate and washings were combined and evaporated to dryness *in vacuo*. A solution of the residue in MeOH was evaporated to dryness giving 197 mg of a cream-colored glass. This material was purified by preparative tlc on two Brinkmann silica gel F-254 plates; $CHCl_3$–MeOH (3:1) was the developing solvent. The product band was eluted with boiling MeOH. Evaporation of the MeOH solution gave the product (β-**13c**) as a white solid: yield 45 mg. This material was identical with that prepared as described in A above.

9-Propenyl-1β-D-ribofuranosylhypoxanthine (β-**15c**).—To an azeotropically dried, refluxing suspension of the mercury salt of 9-propenylhypoxanthine (**9**, 2.04 g, 3.60 mmol) and Celite (2 g) in 250 ml of xylene was added 50 ml of a dry xylene solution of 2,3,5-

TABLE I

ULTRAVIOLET SPECTRAL DATA

Compd	0.1 *N* HCl		pH 7 buffer		0.1 *N* NaOH	
	λ_{max} ($\epsilon \times 10^{-3}$)	λ_{min} ($\epsilon \times 10^{3}$)	λ_{max} ($\epsilon \times 10^{-3}$)	λ_{min} ($\epsilon \times 10^{-3}$)	λ_{max} ($\epsilon \times 10^{-3}$)	λ_{min} ($\epsilon \times 10^{-3}$)
1,3-Dibenzylhypoxanthinium bromide[a]	254 (10.2) 280[b]	236 (7.1)	245[b] 290–300 (0.55)		Unstable	
3-Benzhydryl-1-(tri-*O*-acetyl-β-D-ribofuranosyl)hypoxanthine bromide (7b)	253 (9.78) 280[b] (3.52)	238 (7.98)	255[b] (7.17) 290–310 (3.13)		Unstable	
3,7-Dibenzylhypoxanthine[c]	255.5 (10.1)	237 (7.06)	266 (11.8)	238.5 (5.8)	266 (11.7)	238 (5.5)
1-Benzylhypoxanthine[c]	249 (9.58)	228 (5.60)	251 (9.15)	230 (4.8)	261 (9.75)	239 (4.67)
1-Methylhypoxanthine[d]	249 (9.40)	219 (2.22)	250 (9.00)	224 (2.70)	2.60 (9.60)	236 (3.36)
1-β-D-Ribofurahosylhypoxanthine (13c)	249 (8.95)	223 (3.76)	251 (8.55)	228 (3.91)	261 (8.53)	238 (3.32)
1,9-Dibenzylhypoxanthine[c]	253 (10.6)	232 (6.2)	253 (10.4)	232.5 (5.5)	252 (10.4)	232.5 (5.7)
9-Benzhydryl-1-β-D-ribofuranosylhypoxanthine (β-14c)	253 (12.2)	234 (7.93)	253 (12.2)	234 (7.38)	253 (11.5)	237 (8.93)
1-Methyl-9-propenylhypoxanthine[d]	220 (18.4) 253[b]		225 (24.2) 254[b] 270[b]		225 (25.0) 254[b] 270[b]	
9-Propenyl-1-β-D-ribofuranosylhypoxanthine (15c)	223 (21.4) 253[b]		226 (26.3) 270[b]		226 (25.8) 270[b]	
7-α-D-Ribofuranosylhypoxanthine (α-5c)	252 (9.23)	226 (3.95)	257 (8.40)	229 (3.70)	263 (8.83)	230 (4.00)
7-β-D-Ribofuranosylhypoxanthine (β-5c)	252 (9.10)	226 (4.00)	256 (8.48)	229 (4.07)	263 (9.23)	229 (4.16)

[a] Data from ref 7. [b] Shoulder. [c] Data from ref 2. [d] Data from ref 9.

tri-*O*-benzoyl-D-ribofuranosyl bromide, prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (3.78 g, 7.5 mmol). The mixture was refluxed with stirring for 1 hr and then filtered. The filter cake was washed with boiling $CHCl_3$ (four 25-ml portions). The xylene filtrate was evaporated to dryness *in vacuo;* the residue was dissolved in $CHCl_3$ (100 ml); and this solution was combined with the $CHCl_3$ washings. The solution was washed with 30% KI (two 200-ml portions), then with H_2O (two 200-ml portions), dried over $MgSO_4$, and evaporated to dryness *in vacuo*. A solution of the crude blocked nucleoside was dissolved in MeOH (157.5 ml) containing NaOMe (405 mg, 7.5 mmol), refluxed for 30 min, neutralized with AcOH, and evaporated to dryness. A solution of the residue in 100 ml of H_2O was washed with $CHCl_3$ (50 ml), treated with charcoal, filtered, and then concentrated to 40 ml, whereupon a white solid crystallized, yield 418 mg (18%).

The analytical sample was obtained from a previous run by recrystallization from H_2O. It was dried at 78°: mp 168–170°; δ (ppm) 1.84 (m, CH_3), 3.32 (H_2O), 3.72 (C_5'-H), *ca.* 4.1 (m, C_2'-H, C_3'-H, and C_4'-H), 5.32 (broad m, OH), 6.16 (d, $J_{1'2'}$ = 3 Hz, C_1'-H), 8.37 (C_8-H), 8.75 (C_2-H). The AB portion of the ABX_3 absorption of the propenyl protons is observed as a complex multiplet between 6.1 and 7.4 ppm.

Anal. Calcd for $C_{18}H_{16}N_4O_5 \cdot 0.1H_2O$: C, 50.39; H, 5.23; N, 18.08. Found: C, 50.26; H, 5.46; N, 18.07.

Registry No.—α-5c, 19895-30-8; β-5c, 10280-01-0; β-6a, 20187-88-6; β-6c, 20187-89-7; β-7b, 20290-59-9; 12, 20187-90-0; β-13c, 20187-91-1; β-14c, 20187-92-2; β-15c, 20187-93-3.

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Deuterium Incorporation during the Conversion of 1-Amino-1-deoxy-D-fructose Derivatives to 5-(Hydroxymethyl)-2-furaldehyde[1]

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The formation of Amadori products (1-amino-1-deoxy-2-ketoses) and their subsequent decomposition to melanoidin polymers, furan derivatives, and colored substances is of considerable importance, forming the basis for the syndrome frequently referred to as the nonenzymatic browning reaction.[2] In acidic solution, Amadori products are known[3,4] to undergo decomposition with the production of 2-furaldehyde derivatives as the major monomeric reaction product. It has been suggested[4,5] that the mechanism of this decomposition involves a 1,2 enolization of the Amadori product (I), followed by a dehydration to give the enolic form (II) of a 3-deoxyglycosulose (III), or a Schiff base thereof. In subsequent steps it has been suggested[4] that II or III undergoes further dehydration to the 2-furaldehyde derivative (IV).

In this work, 1-amino-1-deoxy-D-fructose derivatives derived from *p*-toluidine, dibenzylamine, and morpholine were prepared[6] and their decompositon in acidic solution was studied. In both acetic acid and hydrochloric acid, the major monomeric reaction product was 5-(hydroxymethyl)-2-furaldehyde (IV). Yields of IV, determined spectrophotometrically,[7] were variable (see Experimental Section) and the use of acetic acid as a catalyst favored the formation of IV in the over-all reaction. This is in general agreement with the data reported by Gottschalk,[3] for a series of Amadori products composed of weakly basic amines.

In order to investigate the mechanism of the dehydration reaction, the Amadori products were converted

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