TABLE I

ULTRAVIOLET SPECTRAL DATA

| Compd | 0.1 *N* HCl | | pH 7 buffer | | 0.1 *N* NaOH | |
|---|---|---|---|---|---|---|
| | $\lambda_{max}$ ($\epsilon \times 10^{-3}$) | $\lambda_{min}$ ($\epsilon \times 10^{3}$) | $\lambda_{max}$ ($\epsilon \times 10^{-3}$) | $\lambda_{min}$ ($\epsilon \times 10^{-3}$) | $\lambda_{max}$ ($\epsilon \times 10^{-3}$) | $\lambda_{min}$ ($\epsilon \times 10^{-3}$) |
| 1,3-Dibenzylhypoxanthinium bromide[a] | 254 (10.2)<br>280[b] | 236 (7.1) | 245[b]<br>290–300 (0.55) | | Unstable | |
| 3-Benzhydryl-1-(tri-*O*-acetyl-β-D-ribofuranosyl)hypoxanthine bromide (7b) | 253 (9.78)<br>280[b] (3.52) | 238 (7.98) | 255[b] (7.17)<br>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)<br>253[b] | | 225 (24.2)<br>254[b]<br>270[b] | | 225 (25.0)<br>254[b]<br>270[b] | |
| 9-Propenyl-1-β-D-ribofuranosylhypoxanthine (15c) | 223 (21.4)<br>253[b] | | 226 (26.3)<br>270[b] | | 226 (25.8)<br>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.

**Acknowledgments.**—The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations reported. We are also indebted to Mrs. Martha Thorpe of this section for helpful discussions of the pmr data.

# 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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(3) A. Gottschalk, *Biochem. J.*, **52**, 455 (1952).
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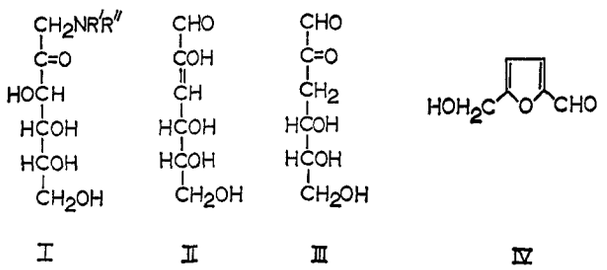
(6) J. E. Hodge and B. E. Fisher in "Methods in Carbohydrate Chemistry," Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press, New York, N. Y., 1963, p 99.
(7) J. F. Harris and coworkers, *Forest Prod. J.*, **X**, 125 (1960).

to IV in deuterium oxide solution using both acetic and hydrochloric acid as catalysts. Estimates of the amount of deuterium incorporated into IV during the conversion were made by proton signal diminution measurements of nmr spectra. A 60-MHz nmr spectra of a pure, crystalline sample of IV showed the aldehyde proton as a singlet at $\delta$ 9.53, the ring proton at position 3 as a doublet centered at $\delta$ 7.59 ($J$ = 3 cps), the ring proton position 4 as a doublet centered at $\delta$ 6.78 ($J$ = 3 cps), and the carbon-bound hydroxymethyl protons as a singlet at $\delta$ 4.69.



I II III IV

A comparison of the signal intensities at $\delta$ 4.69, 6.78, 7.59, and 9.53 indicated that only the latter two signals showed any variation in intensity during the various experiments and was taken to indicate that significant incorporation occurred only at position 3 and at the aldehydic carbon atom. Measurements (Table I) were made by a comparison of signal intensity at $\delta$ 4.69 with that from the proton in question and were determined to be accurate to $\pm 0.1$ atom of deuterium per molecular position.

TABLE I

DEUTERIUM INCORPORATION INTO 5-(HYDROXYMETHYL-2-FURALDEHYDE DERIVED FROM 1-AMINO-1-DEOXY-D-FRUCTOSE DERIVATIVES IN DEUTERIUM OXIDE

| Amine substituent | Acid[b] | Incorporation (%)[c] at aldehyde carbon | Incorporation (%)[c] at position 3 |
|---|---|---|---|
| *p*-Toluidine | Acetic | 23 | 77 |
| *p*-Toluidine | HCl | 0 | 0 |
| Dibenzylamine | Acetic | 75 | 75 |
| Dibenzylamine | HCl | 44 | 0 |
| Morpholine[a] | Acetic | 87 | 87 |

[a] The low yield (less than 4%) of 2-furaldehyde from this compound precluded a measurement in HCl. [b] In all cases, 2 *N* acetic or 1 *N* HCl was used as solvent at a reaction temperature of 100°. [c] These conversions were made in 90% deuterium oxide and the figures are not corrected for water initially contained by the solvent, nor for protons introduced from the acid catalysts or the carbohydrate hydroxyl groups.

Deuterium incorporation at the aldehyde carbon atom of IV is consistent with a reversible enolization of the Amadori product as a first step in the reaction, and differences in the extent of incorporation may be attributed to combinations of amine basicity, acid strength, and the reactivity of the enolic form. While incorporation at position 3 of IV might be due to either reversible 2,3 enolization of I or to reversible equilibration of II and III during the reaction, the finding that, under certain conditions, no detectable incorporation occurs clearly indicates that 3-deoxy-D-glucosulose (III) is not a necessary intermediate in the reaction.

3-Deoxyglucosuloses and their enolic derivatives have been suggested as intermediates in reactions such as the formation of metasaccharinic acids[8] from hexoses in alkaline solution and in the acidic dehydration of hexoses to 5-(hydroxymethyl)-2-furaldehyde,[9] as well as in the reaction considered above. It is interesting to note that recent isotope exchange experiments indicate that III likewise does not appear to be formed during the conversion of D-glucose or D-fructose to IV[10] under a variety of conditions, but the formation of metasaccharinic acid from D-glucose is consistent with III as an intermediate.[11]

The participation of III in the over-all dehydration reaction was further examined by the preparation of III[12] followed by its conversion to IV in deuterium oxide solution at the conditions used for the decomposition of the Amadori products. In both experiments, the resulting IV contained no carbon-bound deuterium.

## Experimental Section

**Materials and Methods.**—Nuclear magnetic resonance spectra were obtained on a Varian A-60 spectrometer at 60 MHz using deuterium oxide as solvent and sodium 3-(trimethylsilyl)propane sulfonate as the internal standard. Standard 5-(hydroxymethyl)-2-furaldehyde (IV) was a commercial sample once recrystallized from ether–hexane. Concentrations of IV in solution were determined by measurement at 283 m$\mu$ using a Beckman DB-G spectrometer on which IV had $\lambda_{max}$ 283 m$\mu$ and $\epsilon$ 17,400. Total absorption at this wavelength was assumed to be due to IV, and calculations were made on the basis of an absorption of 1.0 = 9.1 mg/l. Dehydration products were qualitatively identified by thin layer chromatography using silica gel GF as the support and chloroform–acetic acid (9:1) as irrigant. Purity of Amadori products was determined by paper chromatography using butanol–pyridine–water (6:4:3) as irrigant. Aniline hydrogen phthalate[13] spray reagent was used for both thin layer and paper chromatographic visualizations.

**Preparation of the Amadori Products.**—These compounds were prepared by the procedures described by Hodge and Fisher.[6] The morpholino derivative had mp 143–44°, the *p*-toluidino derivative mp 151–52°, and the dibenzylamino derivative mp 165°. All three compounds ran as single spots on paper chromatograms.

**Reactions of the Amadori Products in Acidic Solution.**—A 3.0-g sample of 1-deoxy-1-toluidino-D-fructose was dissolved in 80 ml of water, and, after the addition of 10 ml of acetic acid, the solution was heated at 100°. At the end of 60 min, when spectral measurements indicated that a maximum yield (33%) had been reached, the solution was cooled to 25°, passed through a column containing Dowex 50 (hydrogen form), and evaporated to dryness at reduced pressure at 40°. The resulting brown solid, when examined by thin layer chromatography, showed the presence of IV as the major reaction product along with traces of an unknown component having an $R_f$ approximately twice that of IV. A uv spectrum of this material in aqueous solution was superimposable with that of a known spectrum of IV, and a nmr spectrum of the material showed signals for all of the protons contained by IV. In this and all subsequent experiments, an approximate 30% loss in IV was observed during isolation. Parallel experiments using solutions of standard IV indicated that this was largely due to irreversible adsorption of IV on the ion-exchange resin. No attempt was made to quantitatively elute the product.

For decompositions in 1 *N* HCl, 3 g of Amadori product was dissolved in 80 ml of acid and heated at 100°. After 2 hr, when the yield of VII from 1-deoxy-1-toludino-D-fructose reached a maximum value of 15%, the solution was cooled and cation exchanged. The effluent was carefully neutralized with 10 *N* NaOH and evaporated to dryness. IV was identified as the major reaction product as above.

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(13) S. M. Partridge, *Nature*, **164**, 443 (1949).

The same procedures were followed for the remaining compounds, in which case the morpholino derivative gave a maximum yield of 3.6% in HCl after 6 hr and 15% in acetic acid after 6 hr, while the dibenzylamino derivative gave 30% yield in 6 hr in acetic acid and 21% in HCl after 2 hr.

**Preparation and Acidic Degradation of 3-Deoxyglucosulose (III).**—This material was prepared from *n*-butyl-D-glucosylamine as described by Kato.[9] A paper chromatographic examination of the syrupy product using *n*-butanol–acetic acid–water (4:1:1) as irrigant showed that it contained largely the glucosulose along with some contaminating glucose. When this preparation was heated at 100° for 1 hr in either 2 *N* acetic acid or 1 *N* HCl and the solutions worked up as described for the Amadori products, all the glucosulose was converted to IV, while parallel experiments showed that the yield of IV from D-glucose was less than 1%.

**Conversions to VII in Deuterium Oxide.**—The Amadori products and III were converted to IV in 90% deuterium oxide solution, either 2 *N* in acetic acid or 1 *N* in HCl, using the same procedures as described above. Following evaporation, the preparations were evaporated to dryness several times from 99% deuterium oxide and the spectra run in the usual way.

**Registry No.**—IV, 67-47-0.

# Studies in the Ganglioside Series. II. Further Application of *N*-Dichloroacetylhexosaminyl Bromides to the Synthesis of Aminosaccharides[1,2]

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The synethesis of galactosaminyl-(1→6)-galactose by two different routes is reported. It involves condensation of bromide III with diketal IV, or with 1,2,3,4-tetraacetylgalactose. The protecting dichloroacetyl group, in addition to its removal by mild alkaline hydrolysis, can be directly converted into the acetyl group by catalytic hydrogenation.

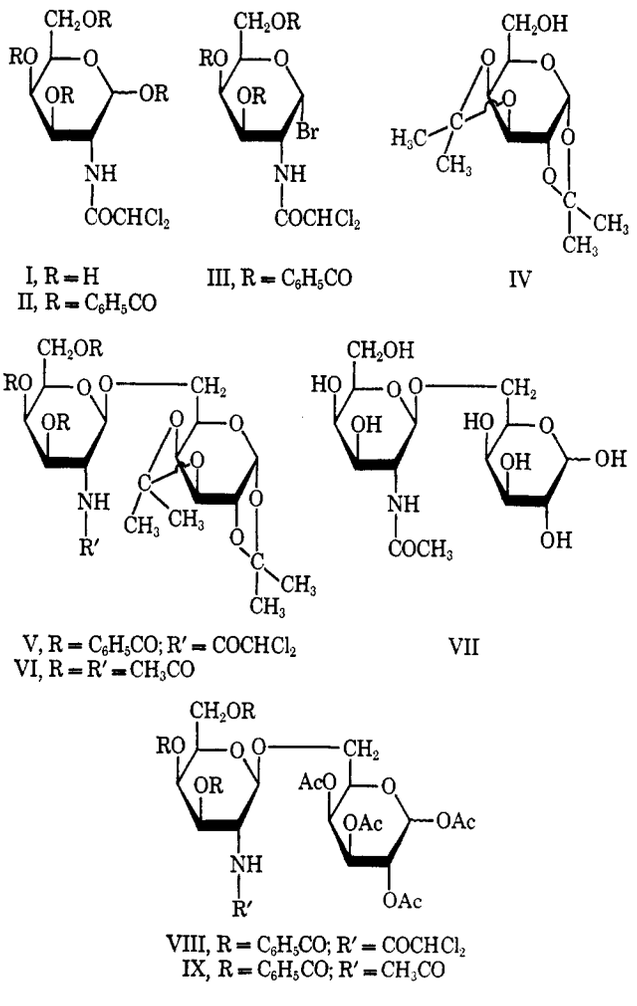
In paper I of this series[3] we described the synthesis of *N*-acetylglucosaminyl-(1→4)-galactose. *N*-Dichloroacetamido-2-deoxy-3,4,6-tri-*O*-benzoylglucopyranosyl bromide used in the Koenigs–Knorr reaction was found to be a reactive and highly stable compound which gave rise to the disaccharide in satisfactory yield. The dichloroacetyl group could be removed by 0.4 *N* aqueous methanolic barium hydroxide at room temperature.

As a preliminary attempt to employ this new type of bromide in the synthesis of galactosaminyl oligosaccharides we have now carried out the synthesis of 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (VII) (Chart I). Oligosaccharides containing the hexosamine (1→6) hexose linkage have been found in human blood group substances.[4,5]

It is noteworthy that, although glucosamine and galactosamine differ only by the steric arrangement at C-4, the latter hexosamine displayed peculiar physical and chemical properties, and we encountered difficulties in the preparation of the key substances. Compound II was obtained in a lower yield as a result of incomplete benzoylation, while the bromide III, although it was chromatographically pure, could not be induced to crystallize. The bromide reacted smoothly with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (IV) in the presence of mercuric cyanide to give V in 90% yield. Lloyd and Roberts[6] have condensed the same diketal with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide and obtained the substituted β-disaccharide in yields of 15–29%, depending on the solvent and the catalyst applied. After debenzoylation and removal of the dichloroacetyl group with barium hydroxide the diketal V was converted into the acteyl derivative VI.

The 1→6 linkage in oligasaccharides is reported to be the least susceptible to acid hydrolysis, in contrast to

CHART I




(1) This work was supported by the National Institutes of Health, Grant No. 425115.

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