[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

# The Amadori Rearrangement under New Conditions and its Significance for Non-enzymatic Browning Reactions[2]

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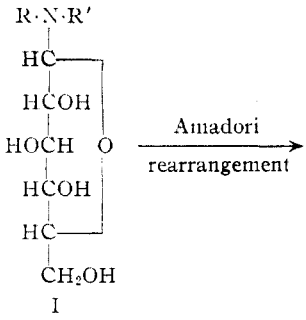
The Amadori rearrangement of glycosylamine derivatives to 1-desoxy-1-amino-2-ketose derivatives occurs (1) slowly in the solid state on storage at 25° and (2) rapidly in hot alcoholic solution in the presence of compounds containing active methylenic hydrogen atoms. These new conditions gave crystalline 1-desoxy-1-amino-2-ketose derivatives from glycosyl derivatives of secondary alkylamines, of primary and secondary aralkylamines, and of a primary aromatic amine. 1-Desoxy-1-piperidino-D-fructose in aqueous solution with amino acids produced brown substances much more rapidly at 25° than did D-glucose or N-D-glucosylpiperidine at the same *p*H. The other desoxyaminoketoses also gave rapid browning reactions with amino acids. Since the Amadori rearrangement occurs spontaneously in N-glycosides in the dry state, and the products of the rearrangement undergo rapid browning reactions with amino acids, a mechanism for non-enzymatic browning in sugar–amine systems based on the Amadori rearrangement is proposed.

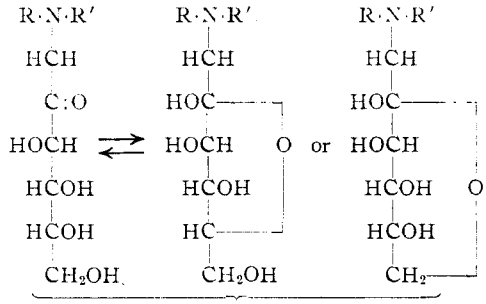
## I. The Amadori Rearrangement

The isomerization of N-substituted glycosylamines (N-glycosides) to 1-desoxy-1-amino-2-ketose (isoglucosamine) derivatives is known as the Amadori rearrangement. Amadori[3] showed that, depending on the manner of heating of D-glucose with a primary arylamine, two different isomers, one labile and one stable, could be isolated. Kuhn, with Dansi,[4] Weygand[5] and Birkofer,[6] identified Amadori's more labile isomer as the N-glucoside (I, R = aryl, R′ = H) and the more stable isomer as an isoglucosamine derivative (II). Whereas both Amadori and Kuhn mentioned reaction conditions which did not involve the addition of acids, Weygand,[7] in a critical review of the rearrangement conditions, was unable to obtain isoglucosamine derivatives consistently from N-D-glucosylarylamines unless catalytic amounts of acid were present.

The Amadori rearrangement has been demonstrated to be general for glycosyl derivatives of primary aromatic amines[6,7]; however, it has been reported not to occur for glucosyl derivatives of piperidine,[6,8] dibenzylamine,[6] and alkylamines in general.[9] Since we have recently shown[10] that Kuhn and Birkofer's "dibenzylamine-N-glucoside"[6] was actually 1-desoxy-1-dibenzylamino-D-fructose (II, R and R′ = $C_6H_5CH_2$–), the restriction of the occurrence of the rearrangement to only primary arylamine-N-glycosides[11] is not valid. We have now found that the Amadori rearrangement occurs for glycosyl derivatives of piperidine, morpholine, diethanolamine and β-phenylethylamine under new conditions that appear generally applicable to all types of glycosylamines.




I




II

The rearrangement of N-D-glucosylpiperidine to 1-desoxy-1-piperidino-D-fructose (DPF) could not be demonstrated under the conditions of Weygand,[7] nor was heating in alcohol[3,6] effective. However, by heating in 1:1 ethanol–ethyl malonate, the rearrangement of both N-D-glucosylpiperidine and N-D-mannosylpiperidine was readily accomplished, yielding crystalline DPF (50%) in each case.

Whereas 1-desoxy-1-dibenzylamino-D-fructose was obtained from D-glucose and dibenzylamine in only 27% yield on heating in alcoholic solution,[10] the addition of ethyl malonate (10% by volume) to the alcoholic solution allowed isolation of the fructose derivative in 89% yield. Similarly, 1-desoxy-1-morpholino-D-fructose (DMF) was obtained in 40% yield from D-glucose (or D-mannose) and morpholine.

Extension of the new isomerization conditions to glycosyl derivatives of aliphatic secondary amines showed first that dimethylamine, diethylamine, diisopropylamine and di-*n*-butylamine do not yield stable crystalline glucosyl derivatives by the usual procedures, although diethanolamine does.[10] (Similar reactivities were found to hold for the aminoly-

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Presented at the 121st National Meeting of the American Chemical Society, Milwaukee, Wis., March, 1952.

(3) M. Amadori, *Atti accad. Lincei*, [6] **2**, 337 (1925); **9**, 68, 226 (1929); **13**, 72, 195 (1931).

(4) R. Kuhn and A. Dansi, *Ber.*, **69**, 1745 (1936).

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

(6) R. Kuhn and L. Birkofer, *ibid.*, **71**, 621 (1938).

(7) F. Weygand, *ibid.*, **73**, 1259 (1940); German Patent 727,402 (Nov. 4, 1942); U. S. Patent 2,354,846 (Aug. 1, 1944).

(8) However, K. Zeile and W. Kruckenberg, *Ber.*, **75**, 1127 (1942), have reported the incidental formation of a trityl derivative of 1-desoxy-1-piperidino-D-xyloketose.

(9) E. Mitts and R. M. Hixon, THIS JOURNAL, **66**, 483 (1944).

(10) J. E. Hodge and C. E. Rist, *ibid.*, **74**, 1494 (1952).

(11) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, p. 386.

sis of esters with the secondary amines used in this work.[12]) The glucosyl derivatives of the fatty secondary amines were very easily hydrolyzed (*cf.*[13]); on the other hand, N-D-glucosyldiethanolamine was relatively quite stable. The rearrangement products from these fatty amine–glucose reactions were sirups, more or less browned, which gave the typical strong reducing behavior of desoxyaminoketoses[6]; but efforts to isolate the latter thus far have been unsuccessful.

Extension of the new reaction conditions to crystalline glucosyl derivatives of primary nonaromatic amines (N-D-glucosyl-*n*-butylamine, N-D-glucosylmonoethanolamine, N-D-glucosylglycine ethyl ester, and D-glucosylamine itself) generally resulted in the isolation of impure, hygroscopic, brown microcrystalline or amorphous products. With N-D-glucosyl-β-phenylethylamine, however, a well-defined hydrated microcrystalline isomerization product was obtained in the presence of phenylacetone, which gave the reducing behavior and browning reactions of 1-desoxy-1-amino-2-ketose derivatives.

In the rearrangement of N-D-glucosyl-*p*-toluidine, the crystalline product, 1-desoxy-1-*p*-toluino-D-fructose, was not obtained in ethanol–ethyl malonate until a small amount of piperidine was added. Also, in general, relatively large amounts (20 to 50% by volume) of the active methylene compound (ethyl malonate, acetylacetone, phenylacetone or diphenylmethane) were necessary to obtain optimum yields of the ketose derivative within 2 or 3 hours. These facts suggest that a temporary condensation of the Knoevenagel type between the ketose derivative and the methylene compound may be involved. The active hydrogen of the methylene compound also would allow formation of the quaternary base cations thought to be intermediates in the rearrangement.[7,11] Dibenzylamine itself evidently yields active hydrogen, since no other methylene compound was necessary for the rearrangement of dibenzylamine–glucose mixtures.[10] Ethyl 2-benzylmalonate was not an effective agent for the rearrangement of N-D-glucosylpiperidine. Other ineffective additives were ethyl oxalate, mineral acids, benzoic acid and succinic acid; on the other hand, malonic acid was quite suitable. Acetylacetone, phenylacetone and diphenylmethane were equally as effective as ethyl malonate, but ethyl acetoacetate reacted differently in that none of the ketose derivative crystallized.

## II. Decomposition of N-Glycosides on Standing

The deterioration with browning of crystalline N-glycosides on standing in moist air is well known[14–16]; nevertheless, the products formed in this deterioration have not been identified. It has been shown that the colored products are fluorescent.[15]

We have isolated two different types of nonfluorescent, crystalline compounds from deteriorated N-glycosides. N-D-Glucosylpiperidine formed a typical red-brown, tarry, heterogeneous mass after standing for 17 months at 25° in a screw-capped vial (not moisture-proof). On extraction of the brown mass with acetone–alcohol, white crystalline DPF (36%) was obtained. In the same way, N-D-glucosyl-*p*-toluidine gave 1-desoxy-1-*p*-toluino-D-fructose. N-D-Galactosylpiperidine also gave a white, crystalline compound which, however, was not 1-desoxy-1-piperidino-D-tagatose, as was expected, but an optically inactive dehydration product of typical reductone character.[17,18] By elementary analysis, the reductone, $C_{11}H_{17}O_3N$, contained 2 moles less of water than N-D-galactosylpiperidine. The same reductone was formed by isomerizing galactosylpiperidine in ethanol–ethyl malonate, by isomerizing glucosylpiperidine with malonic acid, and by heating either galactosylpiperidine or DPF in the dry state; hence, the Amadori rearrangement product is probably a precursor of the reductone. The structure of the new reductone has not yet been determined; however, it is believed to result from an amine-catalyzed sugar dehydration in which all the carbon of the sugar chain is retained.

## III. Mechanism for Non-enzymatic Browning in Sugar–Amine Systems

Both the desoxyaminoketoses and the reductone produced by the spontaneous decomposition of N-glycosides (Part II) underwent rapid browning reactions in aqueous solutions of amino acids at 25° (Fig. 1 and Table I).

Although the browning reactions of DPF with amino acids were obtained at *p*H 8.5, similar browning was shown at a slower rate by DMF at *p*H 7.5, and by DPF with glycine ethyl ester hydrochloride at *p*H 6.3 (initial). All of these reactions were more rapid than those of N-D-glucosylpiperidine with glycine and of D-glucose with glycine at equivalent *p*H. The reductone from galactosylpiperidine and from DPF also showed a browning reaction at *p*H 6, and neutralization of DPF with hydrochloric acid did not stop the browning entirely (Table I). Therefore, the browning reactions of desoxyaminoketoses with amino acids were not limited to alkaline systems; they occur also at a slower rate in neutral and acidic solutions.

The desoxyaminoketose–amino acid systems showed the same phenomena found in natural nonenzymatic browning systems: specifically, (1) substances reducing methylene blue and dichlorophenolindophenol rapidly at *p*H < 8.5 were formed; (2) fluorescent substances were produced; (3) carbon dioxide was liberated from the amino acid *via* the Strecker degradation[19]; (4) the browning reactions were greatly accelerated with increasing *p*H and with increasing temperature; (5) the browning reactions were delayed in the presence of relatively

(12) E. McC. Arnett, J. G. Miller and A. R. Day, THIS JOURNAL, **73**, 5393 (1951).

(13) J. C. Irvine, R. F. Thompson and C. S. Garrett, *J. Chem. Soc.*, **103**, 238 (1913).

(14) F. Weygand, *Ber.*, **72**, 1663 (1939).

(15) H. S. Olcott and H. J. Dutton, *Ind. Eng. Chem.*, **37**, 1119 (1945).

(16) A. Mohammad and H. S. Olcott, THIS JOURNAL, **69**, 969 (1947).

(17) H. v. Euler and H. Hasselquist, "Reductone," Vol. 50 in Sammlung chemischer und chemisch-technischer Vorträge, R. Pummerer, Editor, F. Enke, Stuttgart, Germany, 1950; also H. v. Euler, *Chem. Z.*, **75**, 21 (1951).

(18) F. Petuely and U. Künssberg, *Monatsh.*, **83**, 80 (1952).

(19) A. Schönberg and R. Moubacher, *Chem. Revs.*, **50**, 261 (1952).

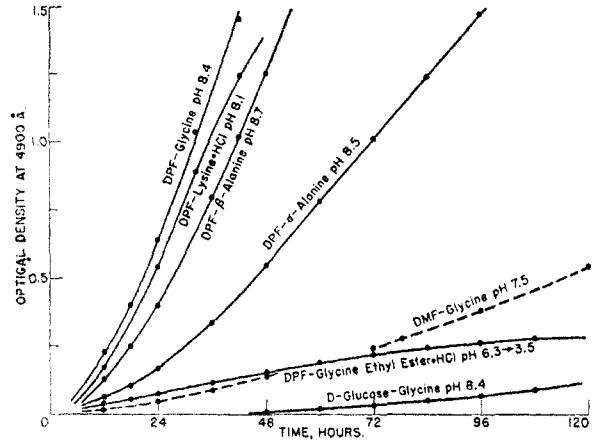



Fig. 1.—Rate of browning of 0.2 *M* aqueous solutions of 1-desoxy-1-piperidino-D-fructose (DPF), 1-desoxy-1-morpholino-D-fructose (DMF) and D-glucose with amino acids (2.0 *M*) at 25°. The solution containing α-alanine was saturated and a small amount remained undissolved.

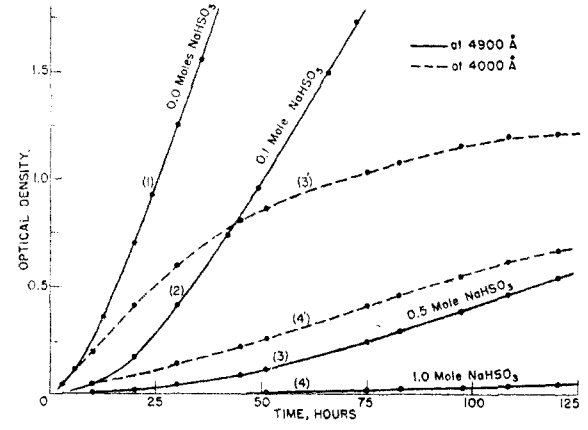



Fig. 2.—Effect of sodium bisulfite in delaying the browning reactions of 1-desoxy-1-piperidino-D-fructose (0.2 *M*) with glycine (2.7 *M*) at 25°, *p*H 7.3–8.3. The molar concentrations of sodium bisulfite are given per mole of DPF.

small amounts of bisulfite and were practically stopped in solutions containing high concentrations of bisulfite; (6) the browning reactions were stopped by hydrogenation of the reaction mixture. Since there is close correspondence between the phenomena exhibited by our model systems of sugars and amines and those of the naturally occurring systems of sugars and amino acids or proteins (*cf.* Rearrangement of N-D-glucosylglycine Ethyl Ester in Part IV), the reactions occurring in each are probably essentially the same.

From our work it is now apparent that non-enzymatic browning in sugar–amine systems can occur through the following reactions: (1) reducing sugars and amino compounds condense to form N-glycosides; (2) the N-glycosides rearrange spontaneously to form desoxyaminoketoses; (3) the desoxyaminoketoses are dehydrated spontaneously to nitrogenous reductones; (4) the labile desoxyaminoketoses (*via* their scission and/or dehydration products) produce the Strecker degradation[19] of amino acids (even in the absence of air) forming aldehydes and carbon dioxide; and (5) the nitrogenous reductones react slowly alone (in the presence of air) and rapidly with amino acids to produce brown pigments. Enediolic reductones[17] easily form α-dicarbonyl compounds by air oxidation, and dienolic reductones[18] form conjugated dicarbonyl compounds[18] which are equally active in producing the Strecker degradation of amino acids.[19] The aldehydes produced by the Strecker degradation condense both with themselves and also with amines,[20] aldimines,[21] amino acids[22] and proteins[22] to produce brown polymers.

Although speculations that the Amadori rearrangement of N-glycosides may be involved in non-enzymatic browning have appeared in the literature,[23–25] the present work demonstrates the actual occurrence of the rearrangement in a sugar–amine browning reaction. Furthermore, the foregoing mechanism for non-enzymatic browning is the first to be presented which is based on the reactions of crystalline intermediates isolated from sugar–amine systems. An elaboration of our theory will be published elsewhere.

TABLE I

COMPARATIVE RATES OF BROWNING OF COMPOUNDS (0.2 *M*[a]) WITH GLYCINE (2.7 *M*) AND ALONE IN WATER AT 25°

| Sugar derivative | *p*H | Optical density, 4900 Å. 1 hour | 1 day | 2 days | 9 days |
|---|---|---|---|---|---|
| Desoxypiperidinofructose | 8.4 | 0.93 | 1.90 | 2.3 | |
| DPF without glycine | 10.4 | .06 | 0.14 | 1.50 | |
| DPF plus HCl (0.2 *M*) | 5.3 | .00 | .01 | 0.06 | |
| Glucosylpiperidine | 8.3 | .02 | .03 | 0.46 | |
| Glucosyldiethanolamine | 8.2 | .01 | .02 | 0.68 | |
| Desoxydiethanolaminofructose[b] | 7.9 | .24 | .42 | 1.25 | |
| Desoxymorpholinofructose | 7.5 | .04 | .13 | 1.10 | |
| DMF without glycine | 9.0 | .00 | .00 | 0.01 | |
| Glucose plus NaOH | 7.4 | .00 | .00 | 0.01 | |
| Glucose plus NaOH | 8.4 | .00 | .01 | 0.43 | |
| Reductone from DPF or galactosylpiperidine[a] | 6.3 | .03 | .05 | 0.30 | |

[a] With the exception of the reductone which was 0.02 *M*. [b] An amorphous gum; the optical densities are corrected for an initial value of 0.12.

## IV. Experimental

The chemicals used were of C.P., Eastman White Label or Matheson Reagent grade, not further purified. The absolute ethanol used was a commercial product (>99.5%) redistilled under anhydrous conditions.

All melting points were determined in capillary tubes and are corrected.

**1-Desoxy-1-piperidino-D-fructose (DPF).**—N-D-Glucosylpiperidine,[10] 5.0 g., was dissolved in hot absolute ethanol (25 ml.) and ethyl malonate (25 ml.) was added. The colorless solution was heated under reflux on a steam-bath for 1.5 hours; it turned progressively yellow, orange, red-orange and deep red. After cooling and filtering, the filtrate was diluted with ether (100 ml.) and cooled at 0° for 24 hours. The crystalline product was filtered, washed with 1:1 ethanol–acetone, and dried; yield 2.6 g. (52%), glittering plates (tinged orange), m.p. 124–126° (dec.). Recrystallized twice from 1:1 methanol–acetone, the colorless plates melted at 127° (dec.); $[\alpha]^{25}D$ −115° (3 min.) →

(20) M. M. Sprung, *Chem. Revs.*, **26**, 297 (1940).
(21) T. M. Patrick, Jr., THIS JOURNAL, **74**, 2984 (1952).
(22) A. Mohammad, H. S. Olcott and H. Fraenkel-Conrat, *Arch. Biochem.*, **24**, 270 (1949).
(23) E. C. Bate-Smith and J. R. Hawthorne, *J. Soc. Chem. Ind.*, **64**, T-297 (1945).
(24) C. H. Lea and R. S. Hannan, *Nature*, **165**, 438 (1950).
(25) A. Gottschalk and S. M. Partridge, *ibid.*, **165**, 684 (1950).

$-50.0°$ (constant after 24 hours, *c* 2.0, pyridine); $[\alpha]^{22}D$ $-57.0°$ (*c* 2.0, *p*H 10.0, water), constant from 5 to 60 minutes after dissolving, decreasing slowly toward zero thereafter.

The isomerization of N-D-mannosylpiperidine,[10] conducted exactly as above, gave DPF (50%), identical in all properties with the compound obtained from N-D-glucosylpiperidine.

*Anal.* Calcd. for $C_{11}H_{21}O_5N$: C, 53.4; H, 8.56; N, 5.66. Found: C, 53.5; H, 8.45; N, 5.70 (Dumas).

Reduction of the heating time or of the concentration of ethyl malonate gave corresponding reductions in the yield. For example, with 17% (by vol.) of ethyl malonate and a heating time of only 45 minutes, N-D-glucosylpiperidine (15%) was crystallized instead of DPF. When ethyl malonate was replaced by phenylacetone (33% by vol.), the yield of DPF was 41%; when acetylacetone (17%) was used, the yield was 34%; with diphenylmethane (50%) the yield was 40%; but when ethyl acetoacetate (50%) was tried, nothing crystallized after seeding with DPF.

DPF was soluble in water and pyridine, moderately soluble in methanol and ethanol, but slightly soluble in acetone, and insoluble in benzene and ether. In 0.05 *N* sodium hydroxide at 25° DPF rapidly reduced methylene blue and dichlorophenolindophenol solution[6] (hereafter abbreviated TR = Tillmans reagent). No immediate reduction of TR was obtained in neutral or acid solution. The test for ene-diols with *o*-dinitrobenzene in alkaline solution[26] also was positive; however, the characteristic violet color, given immediately in the case of L-ascorbic acid, was given after one minute for DPF (changing to a bright yellow on standing 3 hours), but was not given until after 1 hour for N-D-glucosylpiperidine. DPF (also DMF) on heating with ninhydrin reagent in aqueous solution gave a reddish color immediately, whereas the color reaction was delayed for N-D-glucosylpiperidine. DPF reduced Fehling and Benedict solutions in the cold.

Evidence that DPF is easily degraded in alkaline solution follows: In water (*c* 2.0, *p*H 10 → 8) the optical rotation of DPF continually decreased toward zero (after 23 days, $[\alpha]^{25}D$ $-6°$); a yellow color developed in the absence of air (on shaking the solution in the presence of air, it remained colorless), changing to brown on longer standing (Table I); the solution then reduced TR rapidly at *p*H 8.0 and showed a blue fluorescence in ultraviolet light (3650 Å.); upon adding phenylhydrazine acetate to the browned solution (neutralized), an impure reddish precipitate formed in the cold. In 0.1 *N* sodium hydroxide the degradation of DPF to zero optical rotation required only 24 hours, whereas N-D-glucosylpiperidine under the same conditions[10] required 9 days to reach zero rotation.

**Direct Preparation of DPF (or DMF) from D-Glucose.**—Anhydrous D-glucose, 90 g. (0.50 mole) and piperidine, 57 g. (0.67 mole), were stirred mechanically at 60–75° until a homogeneous amber sirup was obtained (20 minutes). The heating-bath was removed and malonic acid, 18 g. (0.17 mole), was added slowly to the stirred sirup over a 10-minute period. The temperature rose to 80°, and the color of the sirup deepened to a reddish hue. After stirring 5 minutes longer, ethanol (70 ml.) was added, then the solution was heated at 75° for 30 minutes. Adding acetone (70 ml.) and seed crystals produced, after 1 hour at 25°, the first batch of DPF, 28 g., m.p. 123–125° (dec.). Storing and filtrate and washings at 0° produced (after several weeks) a second batch, 9 g., m.p. 121–124° (dec.). (Reheating the mother liquors at 95–100° was required to produce further crystallization at 0° without long waiting periods); total crude yield 37 g. (30%). Recrystallization from hot ethanol (200 ml.) plus acetone (200 ml.) gave DPF as colorless, glistening plates, 33 g. (27%), m.p. 126–127° (dec.).

Surprisingly, the filtrate from the second batch of crystals, after concentration and storage at 0° for 4 months, gave the reductone compound (6 g.) described below.

**1-Desoxy-1-piperidino-D-fructose Phenylhydrazone.**—A solution of DPF, 1.0 g. (4.0 mmoles) and phenylhydrazine, 0.48 g. (4.4 mmoles), in methanol (13 ml.) was heated on the steam-pot and evaporated to a crystalline residue. Recrystallized from ethanol, colorless platelets, 0.35 g., m.p. 175° (dec.), were obtained, $[\alpha]^{25}D$ $-41.0°$ (*c* 2.0, pyridine). The hydrazone in 0.1 *N* potassium hydroxide in methanol gave no reduction of TR.

*Anal.* Calcd. for $C_{17}H_{27}O_4N_3$: C, 60.5; H, 8.07; N, 12.5. Found: C, 60.6; H, 7.70; N, 12.5.

**1-Desoxy-1-piperidino-D-fructose Oxime.**—A solution of hydroxylamine (0.9 g.) in absolute ethanol (20 ml.)[27] was added to a hot solution of DPF (4.0 g.) in absolute ethanol (12 ml.). The combined solutions were boiled under reflux for 15 minutes, filtered and concentrated *in vacuo* to a sirup. The sirup was fractionally precipitated from ethanol–ether, yielding 1.5 g. (35%) of crude crystalline oxime, m.p. 135–140°. Two recrystallizations from ethanol–ether gave the pure compound, m.p. 141–142°, $[\alpha]^{25}D$ $-31°$ (3 minutes) → $-14°$ (constant, 1 to 7 days, *c* 2.0, water). The aqueous solution was strongly alkaline and turned yellow-brown in the polarimeter tube. The compound, when dissolved in 0.1 *N* sodium hydroxide at 25°, gave no immediate reduction of TR.

*Anal.* Calcd. for $C_{11}H_{22}O_5N_2$: C, 50.4; H, 8.45; N, 10.7. Found: C, 50.1; H, 8.22; N, 10.6.

**1-Desoxy-1-piperidino-D-fructose Oxime Hydrochloride.**—Hydroxylamine hydrochloride, 0.75 g. (11 mmoles), DPF, 2.5 g. (10 mmoles), and absolute ethanol (40 ml.) were heated together under reflux for 1 hour. The colorless solution was concentrated *in vacuo* to a thick sirup which was kept at 0° until crystalline; yield 2.4 g. (80%), m.p. 164–166°. Recrystallized from ethanol (30 ml.), the pure oxime hydrochloride was obtained, m.p. 165–166° (gas evolved at 172–175°), $[\alpha]^{25}D$ $-44°$ (*c* 2.0, water)

*Anal.* Calcd. for $C_{11}H_{23}O_5N_2Cl$: C, 44.2; H, 7.76; N, 9.38; Cl, 11.9. Found: C, 44.4; H, 7.92; N, 9.48; Cl, 11.8.

**Isopropylidene Derivative of DPF.**—A mixture of dry acetone containing 1.2% hydrogen chloride by weight (200 ml.) and DPF, 2.5 g. (10 mmoles), was kept at 0° for 3 hours (with frequent shaking) and at 25° for 18 hours. The crystalline product, washed with acetone and dried, weighed 1.8 g., m.p. 167° (dec.). The filtrate, after concentration *in vacuo*, yielded 0.3 g. more of the same product. After recrystallization from absolute ethanol, 1.8 g. (56%) of the pure compound was obtained as colorless prismatic needles, m.p. 169° (dec.), $[\alpha]^{25}D$ $-84.3°$ (*c* 2.0, water) without mutarotation. In 0.1 *N* sodium hydroxide at 25°, the derivative still reduced TR.

*Anal.* Calcd. for the hydrochloride of a monoisopropylidene derivative, $C_{14}H_{26}O_5NCl$: C, 51.9; H, 8.09; N, 4.33; Cl, 11.0. Found: C, 51.7; H, 8.05; N, 4.33; Cl, 11.3.

*Periodate Oxidation.*[28]—The isopropylidene hydrochloride derivative of DPF (0.500 mmole) in 100 ml. of aqueous solution containing sodium metaperiodate (1.50 mmoles) consumed 1.05 moles of periodate per mole in 3 hours at 2° (*p*H 4.5). The value was unchanged after 6 hours. At the same time, 0.66 mole of acid was produced per mole of compound.

**Hydrogenation of DPF.**—A solution of DPF, 2.5 g. (10 mmoles), in 50% aqueous methanol was hydrogenated in a steel bomb (300-ml. capacity)[29] with Raney nickel (4 g.).[30] The bomb was heated in a rocking oven at 55° for 10 hours. After filtration, concentration *in vacuo* (with the addition of ethanol and benzene), and refrigeration, a white, crystalline product, 2.2 g. (88%), m.p. 107–111°, was obtained. Recrystallization from absolute ethanol (15 ml.) gave 1.2 g. of colorless prismatic needles, m.p. 116°, $[\alpha]^{26}D$ $-17.2°$ (*c* 4.0, pyridine). A mixture with N-1′-D-sorbitylpiperidine[10] gave no lowering of the melting point. The mother liquor gave 0.4 g. more of the same compound, m.p. 113–116°, total yield 1.6 g. (64%). Hence, DPF gave predominantly the glucamine, whereas hydrogenation of 1-desoxy-1-arylamino-D-fructoses gave predominantly mannamines.[6,31]

(26) W. R. Fearon and E. Kawerau, *Biochem. J.*, **37**, 326 (1943).

(27) A. Wohl, *Ber.*, **26**, 730 (1893).

(28) The method of Fleury and Lange was used as described by Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 361. Acid was determined by a modification of the method of Halsall, Hirst and Jones, *J. Chem. Soc.*, 1427 (1947).

(29) H. Adkins, "Reactions of Hydrogen, etc.," University of Wisconsin Press, Madison, Wisconsin, 1937, p. 29.

(30) R. Mozingo, in "Organic Syntheses," **21**, 15 (1941).

(31) F. Weygand, *Ber.*, **73**, 1278 (1940).

When the hydrogenation was repeated in absolute methanol (for 5 hours at 55°), 50% of the starting material, DPF, was recovered unchanged.

**1-Desoxy-1-dibenzylamino-D-fructose.**—α-D-Glucose, 5.0 g. (28 mmoles), and dibenzylamine, 8.1 g. (41 mmoles), were mixed and 150 ml. of a solution of ethyl malonate (10% by volume) in absolute ethanol was added. The mixture was heated under reflux for 2 hours on the steam-bath. The sugar dissolved after ½ hour. The golden yellow solution was cooled, filtered and stored at 0° for 20 hours, yielding 8.9 g. (89%) of 1-desoxy-1-dibenzylamino-D-fructose,[10] m.p. 161–162° (dec.). Recrystallized twice from methanol, the pure compound melted at 163° (dec.).

**1-Desoxy-1-morpholino-D-fructose (DMF).**—By the procedure next above, using 50 ml. of 1:1 ethanol–ethyl malonate and diluting with ether (100 ml.) after the 2-hour reaction period, both D-glucose and D-mannose yielded DMF (40%), m.p. 145–146° (dec.). Recrystallized twice from methanol, colorless rhombohedric tablets were obtained, m.p. 147° (dec.), $[\alpha]^{25}D$ −65.4° (*c* 2.0, *p*H 8.7, water), $[\alpha]^{25}D$ −117° (12 minutes) → −50.8° (constant after 48 hours, *c* 2.0, dry pyridine). The compound in 0.1 *N* sodium hydroxide at 25° rapidly reduced TR.

*Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.2; H, 7.68; N, 5.62. Found: C, 48.1; H, 7.55; N, 5.63.

By the practical preparative procedure given for DPF, DMF was obtained from D-glucose in 55% of the theoretical yield, m.p. 144–145° (dec.).

**1-Desoxy-1-morpholino-D-fructose Phenylhydrazone.**—By the procedure given for DPF, the phenylhydrazone of DMF was prepared, m.p. 164° (dec.), $[\alpha]^{25}D$ −58.4° (*c* 1.0, pyridine).

*Anal.* Calcd. for $C_{16}H_{25}O_5N_3$: C, 56.6; H, 7.43; N, 12.4. Found: C, 56.8; H, 7.28; N, 12.9.

**Periodate Oxidation[28] of DPF and DMF.**—DPF (0.500 mmole) was neutralized with hydrochloric acid (0.500 mmole). In the presence of sodium metaperiodate (3.00 mmoles) in 100 ml. of aqueous solution (*p*H 4.5), DPF consumed after 3, 6 and 24 hours, 4.25, 4.25 and 4.28 moles of periodate per mole. At the same time, there was produced 2.82, 2.90 and 3.03 moles of acid per mole of DPF. The results were as expected, presuming that only formic acid was titrated; piperidinoacetic acid, the other expected product, is a neutral compound.

In the same way, DMF (0.188 mmole), HCl (0.20 mmole), and sodium metaperiodate (0.957 mmole) in 100 ml. solution (*p*H 4) gave after 2, 5, 13 and 48 hours: 3.80, 3.85, 3.91 and 4.04 moles of periodate consumed per mole of DMF.

**Paper Chromatography of DPF and DMF.**—Solutions of DPF and DMF in methanol were spotted on Whatman No. 1 filter paper and developed (descending) with *n*-butanol–methyl cellosolve–water (2:1:1) at 25° for 17 hours. Spraying with ammoniacal silver nitrate, drying and heating the paper at 75–80° gave a single spot for DMF (DMF gives a nearly constant optical rotation in water), $R_F$ 0.64, and a long continuous streak for DPF (DPF gives a mutarotation in water), $R_F$ 0.47 → 0.78.

**Acetylation of Desoxyaminofructoses.**—Attempts at acetylation of DPF, DMF and 1-desoxy-1-dibenzylamino-D-fructose in pyridine–acetic anhydride by the usual procedure resulted always in the isolation of dark red-brown sirups, very soluble in both water and organic solvents. However, from the acetylation of 1-desoxy-1-dibenzylamino-D-fructose in pyridine–acetic anhydride at 0°, a white, crystalline triacetyl derivative (6%), m.p. 120–121° (dec.), was obtained from the dark sirup as a by-product, $[\alpha]^{25}D$ −102° (*c* 0.4, chloroform). The triacetate in 0.1 *N* potassium hydroxide in methanol at 25° rapidly reduced TR.

*Anal.* Calcd. for $(C_7H_7)_2N{\cdot}C_6H_8O_5(COCH_3)_3$: C, 64.3; H, 6.43; N, 2.89; $COCH_3$, 26.6. Found: C, 64.2; H, 6.46; N, 3.00; $COCH_3$, 28.2.

**1-Desoxy-1-diethanolamino-D-fructose.**—N-D-Glucosyldiethanolamine,[10] was treated as described for N-D-glucosylpiperidine with ethyl malonate, acetylacetone, phenylacetone and diphenylmethane. In each case a taffy-like, stiff yellow gum was isolated after repeated precipitations from ethanol into ether; no crystals were obtained. The products retained ethanol and were very hygroscopic. The Amadori rearrangement evidently occurred, however, for the purified product in 0.1 *N* sodium hydroxide solution reduced TR rapidly at 25°. Also, the rate of browning in 20% aqueous glycine solution was several times more rapid than that of N-D-glucosyldiethanolamine under the same conditions (Table I).

*Anal.* Calcd. for $C_{10}H_{21}O_7N{\cdot}{}^1/_5C_2H_5OH{\cdot}{}^1/_4H_2O$: C, 44.5; H, 8.1; N, 5.0; $OC_2H_5$, 3.2. Found: C, 44.5; H, 7.9; N, 4.3; $OC_2H_5$, 3.2.

**N-(3-Methyl-D-glucosyl)-piperidine.**—This new compound was prepared by the procedure previously reported for N-D-glucosylpiperidine.[10] 3-Methyl-D-glucose, m.p. 161–163°, 0.97 g. (5 mmoles), and piperidine, 0.68 g. (8 mmoles), gave colorless prismatic needles, 0.50 g. (38%). Recrystallized twice from 1:9 ethanol–ether, the compound melted at 130–131°, $[\alpha]^{24}D$ +8.8° (*c* 1.3, pyridine) without mutarotation. The compound in dilute alkali at 25° did not reduce TR.

*Anal.* Calcd. for $C_{12}H_{23}O_5N$: N, 5.36; $OCH_3$, 11.9. Found: N, 5.29; $OCH_3$, 11.9.

**Amadori Rearrangement of 2-Methyl- and 3-Methyl-D-glucosyl Derivatives of Piperidine.**—N-(2-Methyl-D-glucosyl)-piperidine,[32] 0.10 g., was subjected to the conditions used for the rearrangement of N-D-glucosylpiperidine. But little color developed, and the starting material (70%) was recovered unchanged. The experiment was repeated with the 3-methyl derivative described above. A sirupy product was obtained which, in 0.1 *N* sodium hydroxide at 25°, reduced TR, indicating that the Amadori rearrangement had perhaps occurred.

**1-Desoxy-1-*p*-toluino-D-fructose.**—The rearrangement of N-D-glucosyl-*p*-toluidine,[14] 10.4 g., was accomplished as for N-D-glucosylpiperidine, except that piperidine, 0.5 ml., was added to the ethanol–ethyl malonate solution before heating; yield 5.5 g. (53%) of crude product, m.p. 145–148°. After recrystallization from ethanol (80 ml.), the pure compound, 3.9 g. (38%), melted at 153–154° (dec.), $[\alpha]^{25}D$ −64° (initial) → −21° (final, *c* 1.1, pyridine).[6,7] In 0.1 *N* sodium hydroxide, the product reduced TR rapidly, whereas the starting material gave no immediate reduction of the dye. When the experiment was run without adding piperidine, none of the fructose derivative crystallized.

**N-D-Glucosyl-β-phenylethylamine Monohydrate.**—α-D-Glucose, 9.0 g. (50 mmoles), and β-phenylethylamine, 12.1 g. (100 mmoles), yielded, after warming for 10 minutes on the steam-bath, extracting once with ether (50 ml.) to remove the excess amine and diluting the sirupy residue with ether (30 ml.) plus water (1 ml.), 13.2 g. (88%) of a white crystalline compound, m.p. 90–91°. Recrystallized from ethanol (50 ml.), the pure compound, 11.3 g. (75%), melted at 92–93°, $[\alpha]^{25}D$ −25° (4 minutes) → +2° (constant after 2 days, *c* 4.0, pyridine).

The compound in 0.1 *N* sodium hydroxide did not reduce TR at 25°. It was noticeably more stable than N-D-glucosyl-*n*-butylamine hydrate[9,10,16] on standing at 25°.

*Anal.* Calcd. for $C_{14}H_{21}O_5N{\cdot}H_2O$: C, 55.8; H, 7.69; N, 4.65. Found: C, 55.9; H, 7.69; N, 4.51.

**Rearrangement of N-D-Glucosyl-β-phenylethylamine Monohydrate.**—The compound obtained in the paragraph next above, 1.0 g., was dissolved in warm absolute ethanol (15 ml.), phenylacetone (5 ml.) was added, followed by morpholine (0.3 ml.). After heating for 40 minutes in the steam-pot, the golden orange solution was poured into ether (300 ml.), precipitating an oil which changed to a fine microcrystalline precipitate on standing at 0° overnight. The supernatant was decanted (inside a dry transfer box) and the crystals were washed twice with ether. Finally the hygroscopic, almost white product was transferred to a weighing bottle and dried *in vacuo* (1 mm.) over phosphorus pentoxide for 2 weeks; yield 0.25 g., m.p. 75–80°, $[\alpha]^{25}D$ −10° (constant after 5 minutes, *c* 0.5, pyridine).

*Anal.* Calcd. for $C_{14}H_{21}O_5N{\cdot}2{}^1/_2H_2O$: C, 51.2; H, 7.98; N, 4.27. Found: C, 51.2; H, 7.71; N, 4.33.

The hydrated compound lost 2.6% on drying over boiling acetone in an Abderhalden apparatus (< 1 mm. pressure) for 5 hours, without melting (calcd. for the loss of $^1/_2H_2O$: 2.75%); then, drying over boiling ethanol for 3 additional hours, the loss increased to 3.6%, but the compound melted to a glassy sirup and could not be further dried. In 0.1 *N* sodium hydroxide at 25° the substance reduced TR in the manner of desoxyaminoketose derivatives; it also underwent browning in 20% aqueous glycine at 25°.

(32) J. E. Hodge and C. E. Rist, THIS JOURNAL, **74**, 1498 (1952).

Since the compound analyzes for an isomer of N-D-glucosyl-β-phenylethylamine, except for water of hydration, it is considered to be 1-desoxy-1-β-phenylethylamino-D-fructose.

**Rearrangement of N-D-Glucosylglycine Ethyl Ester.**—N-D-Glucosylglycine ethyl ester,[33] m.p. 109–110°, $[\alpha]^{25}D$ −8° (initial) → +2° (after 24 hours, *c* 3.0, absolute ethanol) (5.0 g.) was isomerized (A) as for N-D-glucosylpiperidine, except that piperidine (2 drops) was added to the ethanol-ethyl malonate solution, and (B) as for N-D-glucosyl-β-phenylethylamine above. The reaction was complicated by partial hydrolysis of the ester linkage; yields 2.5–3.0 g. of microcrystalline, hygroscopic, orange powder, m.p. 60–75°, $[\alpha]^{25}D$ (A) −13° (4 minutes) → −8° (constant, 1–4 days, *c* 0.7, absolute ethanol); (B) −5° (5 minutes) → −2° (constant, 1–7 days, *c* 1.2, absolute ethanol). In 0.1 *N* sodium hydroxide at 25° both A and B rapidly reduced TR; they also showed enhanced browning after 48 hours standing in 20% aqueous glycine solution at 25°, *p*H 6.0. N-D-Glucosylglycine ethyl ester under the same conditions, but at *p*H 8, showed no browning after 48 hours. Aqueous solutions of A showed a strong blue fluorescence in ultraviolet light. Both products were dried over phosphorus pentoxide *in vacuo* at 25° for 2 weeks.

*Anal.* Calcd. for $C_{10}H_{19}O_7N$: C, 45.3; H, 7.2; N, 5.3; $OC_2H_5$, 17.0. Found: (A) C, 45.8; H, 7.0; N, 4.5; $OC_2H_5$, 15.7. (B) C, 45.9; H, 7.3; N, 5.1; $OC_2H_5$, 13.3.

**Rearrangement of N-D-Glucosylethanolamine.**—Following the isomerization procedure for N-D-glucosylpiperidine, adding piperidine (3 drops) as a catalyst, N-D-glucosylethanolamine[10] gave, with both ethyl malonate and acetylacetone, light brown, hygroscopic powders, 2.2 g. (44%); $[\alpha]^{22}D$ −36° (using ethyl malonate); −39° (using acetylacetone); (*c* 1.8, water). The products, when dissolved in 0.1 *N* sodium hydroxide at 25°, quickly reduced TR.

**Autoisomerization of N-D-Glucosylpiperidine and N-D-Glucosyl-*p*-toluidine.**—N-D-Glucosylpiperidine[10] was stored in a screw-capped vial (not moisture-proof) for 17 months at 25 ± 5°. It slowly turned brown and finally coalesced to a spongy heterogeneous mass containing oily droplets. The brown material, 5.3 g., was almost completely dissolved in methanol (25 ml.), then an equal volume of acetone was added. After storage at 0° the crystalline precipitate was filtered, washed with acetone and dried; yield 1.9 g. (36%), m.p. 125–127° (dec.). After recrystallization from ethanol (12 ml.), the product was identical in all its properties with 1-desoxy-1-piperidino-D-fructose described above. When heated with phenylhydrazine in methanol, the product gave 1-desoxy-1-piperidino-D-fructose phenylhydrazone, m.p. 175° (dec.). Two other samples of N-D-glucosylpiperidine changed in the same way on long standing at 25° to yield crystalline DPF.

N-D-Glucosyl-*p*-toluidine,[4] m.p. 110–112°, gave after 9 months at 25 ± 5°, 1-desoxy-1-*p*-toluino-D-fructose (49%), m.p. 150° (dec.). Recrystallized from ethanol, white needles (37%), m.p. 153–154° (dec.), were obtained. The compound in 0.1 *N* sodium hydroxide at 25° quickly reduced TR and was identical in all its properties with 1-desoxy-1-*p*-toluino-D-fructose described above.

**Spontaneous Dehydration of N-D-Galactosylpiperidine to a Crystalline Compound of Reductone Character.**—N-D-Galactosylpiperidine[10] stood in a screw-capped vial in a desiccator over anhydrous calcium chloride for 2 years and 5 months. The white crystals coalesced to a dark, rust-brown heterogeneous mass. The mass, 10.2 g., was extracted with 1:1 methanol–acetone, yielding 1.7 g. (20%) of fine crystals, discolored tan, m.p. 225–227° (after continuous decomposition above 200°). Recrystallized twice from methanol, the pure white stable compound decomposed slowly above 210°, finally melting at 230–232°.

*Anal.* Calcd. for $C_{11}H_{17}O_8N$: C, 62.5; H, 8.11; N, 6.63; mol. wt., 211. Found: C, 62.6; H, 8.08; N, 6.59 (Dumas); mol. wt., 239 (Signer).

A saturated aqueous solution of the compound at 25° (*c* 0.3, *p*H 6.6) was optically inactive. The solution reduced acid silver nitrate, mercuric chloride and TR rapidly at 25°. A positive ene-diol test with *o*-nitrobenzene[26] was obtained at once on adding alkali, as for L-ascorbic acid. Also, like L-ascorbic acid, the compound could be titrated with iodine in acid solution, giving a sharp end-point after two equivalents of iodine per mole of compound were reduced. This behavior classifies the compound as a reductone.[17] An aqueous solution of the compound gave no deep color reaction with ferric chloride (like L-ascorbic acid but unlike triose reductone). When heated with phenylhydrazine hydrochloride in acetate solution, the reductone was recovered unchanged.

The reductone was not hydrogenated at atmospheric pressure in glacial acetic acid or water at 28° with a reduced platinum oxide catalyst (semi-micro apparatus). However, in 0.01 *N* sulfuric acid under the same conditions, 3.3 moles of hydrogen was consumed per mole of compound within 2 hours.

Between the limits of 2200 and 4000 Å., the reductone in water, *p*H 6, gave a single absorption peak at 3085 Å. ($E^{1\%}_{1\,cm}$ 1490). In 0.008 *N* hydrochloric acid the absorption was but little changed ($\lambda_{max}$ 3060 Å., $E^{1\%}_{1cm}$ 1220); on the other hand, in 0.008 *N* sodium hydroxide the absorption nearly disappeared ($\lambda_{max}$ 3150 Å., $E^{1\%}_{1\,cm}$ 14; an end absorption at 2200 Å. was evident, however) and could not be regenerated by adding acid. When the reductone was dehydrogenated by adding two equivalents of iodine per mole in 0.002 *N* hydrochloric acid, a single absorption peak was obtained ($\lambda_{max}$ 2280 Å., $E^{1\%}_{1\,cm}$ 2970). When approximately one equivalent of iodine was added per mole of reductone in 0.003 *N* hydrochloric acid, about half of the absorption remained at 3070 Å. ($E^{1\%}_{1\,cm}$ 540) and the peak corresponding to the dehydro form was only half developed at 2280 Å. ($E^{1\%}_{1\,cm}$ 1440). The reductone apparently was easily oxidized by air, for after a dilute neutral solution of the reductone in water had stood at 25° for 20 hours, the peak at 3085 Å. was considerably diminished and a broad double peak appeared at 2300–2400 Å. corresponding in part to the dehydro form.

Chromatography of the reductone, conducted exactly as described above for DPF and DMF, gave a single spot after spraying with ammoniacal silver nitrate and drying at room temperature ($R_F$ 0.75–0.79). On heating the paper no other spots developed.

The reductone, dissolved alone in methanol or pyridine (*c* 1.0) or water (*c* 0.3) produced a slow browning reaction on standing at 25°. All solutions were optically inactive. In 20% aqueous glycine a rapid browning reaction was produced (Table I).

**Preparation of the Reductone by Pyrolysis of DPF or N-D-Galactosylpiperidine.**—DPF, 6.0 g., in a tared vial (28 × 100 mm.) was heated in an Abderhalden drying apparatus over boiling toluene for 5 hours (< 1 mm. pressure, phosphorus pentoxide desiccant). The crystals slowly turned brown then coalesced to a red-brown sirup. The sirup boiled, yielding a colorless crystalline sublimate, 0.75 g., a clear sirup distillate, 0.85 g., and a very dark-brown residue, 3.3 g. The sublimate of large deliquescent crystals was collected, recrystallized thrice from dry acetone and dried over phosphorus pentoxide, m.p. 103–104°. A mixture with an authentic sample of piperidine acetate, m.p. 103–105°, also melted at 103–104°.

*Anal.* Calcd. for $C_5H_{11}N{\cdot}CH_3COOH$: $CH_3COOH$, 41.4. Found: $CH_3COOH$ (Elek), 41.3.

The piperidine acetate was further characterized by converting it with picric acid to piperidine picrate, m.p. 150–151° (identified by mixed m.p. with an authentic sample). On warming with picric acid, the odor of acetic acid was detected.

The crystalline reductone, 0.4 g. (8%), was isolated from the residue in the manner described in the preceding section. Recrystallized from methanol, the reductone melted at 229–232° (with slow decomposition above 210°), and a mixed m.p. with the reductone obtained from galactosylpiperidine without heating was not changed.

In the same way, N-D-galactosylpiperidine,[10] on heating *in vacuo* at 78° for 5 hours (or at 100° for 2 hours), gave the same crystalline reductone, m.p. 229–232° (dec.), in 5–10% yields. A sublimate of piperidine acetate and a clear sirupy distillate also were formed as during the pyrolysis of DPF.

**Preparation of the Reductone by Isomerization of N-D-Galactosylpiperidine in Ethanol-Ethyl Malonate.**—N-D-Galactosylpiperidine was prepared by the method pre-

(33) M. L. Wolfrom, R. D. Schuetz and L. F. Cavalieri, THIS JOURNAL, **71**, 3518 (1949).

viously published[10] and recrystallized twice from methanol, m.p. 126–127° (dec.), $[\alpha]^{25}D$ −1° (4 min.) → −25° (1–4 hours; *c*, 2.0, pyridine), $[\alpha]^{25}D$ +16° (2.5 min.) → −7° (2–24 hours, *c* 1.0, absolute methanol). *Anal.* Calcd. for $C_{11}H_{21}O_5N$: C, 53.4; H, 8.56; N, 5.66. Found: C, 53.5; H, 8.60; N, 5.82. This product, 5.0 g., was isomerized exactly as described for N-D-glucosylpiperidine. Only a small amount of sirup was precipitated from the ethereal solution after 2 months at 0°. The supernatant was decanted, seeded with a few crystals of the reductone from galactosylpiperidine, and kept in a closed flask at 25°. Within 4 months several large prismatic crystals (0.4 g.) formed. These were recrystallized from ethanol, yielding 0.2 g. of white crystals, m.p. 230–232° (dec.), identical in all properties with the reductone described above.

**Decomposition of N-D-Glucosylmonoethanolamine to a Melanoidin of Reductone Character.**—N-D-Glucosylmonoethanolamine,[10] 30.5 g., was kept in a screw-capped bottle in a desiccator over calcium chloride at 25° for 20 months. The crystals slowly turned brown and coalesced to a very dark brown tar. The tar was extracted 4 times with 1:4 methanol–acetone, then the residue was dissolved in methanol (20 ml.) and acetone (80 ml.) was stirred into the solution. The precipitate thus obtained was reprecipitated twice in the same way, yielding 18 g. (59%) of a hygroscopic, chocolate-brown, melanoidin-like powder. An aqueous solution of the product (*c* 1.0, *p*H 6.7) reduced TR rapidly at 26° (acid silver nitrate also was reduced), indicating a reductone structure in the melanoidin. Although the product was kept *in vacuo* over calcium chloride for a month, it gave a small percentage of methoxyl.

*Anal.* Calcd. for glucosylethanolamine, $C_8H_{17}O_6N$: C, 43.1; H, 7.67; N, 6.27. Found: C, 50.3; H, 7.45; N, 5.95; $OCH_3$, 1.4.

**Strecker Degradation of Amino Acids.**—By Procedure (b) of Schönberg, *et al.*,[34] the degradation of DL-α-aminophenylacetic acid to benzaldehyde by DPF was effected in the absence of air. Benzaldehyde was isolated as the phenylhydrazone, m.p. 152–154° (turning orange-red in daylight), in 25% of the theoretical yield (based on the amount of amino acid dissolved). Qualitative tests showed, by characteristic odors of the aldehydes, that DPF degraded leucine to isovaleraldehyde and valine to isobutyraldehyde. Carbon dioxide was liberated in all cases.

**Rate of Browning of Desoxyaminofructoses with Amino Acids.**—The desoxyaminofructose, the N-glucoside or D-glucose (2.00 mmoles) and the amino acid (20 mmoles, except where noted otherwise) were dissolved in water (10 ml.) in optically matched tubes (18 × 150 mm.). The corked tubes were stored in the dark at a constant temperature of 25°. Blanks contained equal concentrations of the amino acid. Optical density measurements were made in a spectrophotometer at 4900 Å. and at 4000 Å. Although solutions beginning to turn brown gave higher absorptions at the lower wave length, the dark-brown solutions gave higher absorptions at the higher wave length. Increases in optical density at 4000 Å. were evident before visible browning began. The results are recorded in Figs. 1 and 2, and Table I.

In water alone at *p*H 10 → 8, DPF showed appreciable browning at 25° (note that DMF showed much less browning at *p*H 9 → 7.8; see Table I), but this browning was much slower than that obtained in the presence of amino acids at lower *p*H. The increase in lability of desoxyaminofructoses with increasing *p*H is therefore quite marked.

As the browning reactions proceeded, the solutions developed a blue fluorescence in ultraviolet light, 3650 Å. (also true for the colorless solutions containing bisulfite[35]) and also a reducing power toward TR (without the addition of alkali). The *p*H had decreased only a few tenths of a unit after 4 days of browning in the glycine, alanine and lysine hydrochloride solutions; but in the case of glycine ethyl ester hydrochloride and DPF (Fig. 1), the *p*H dropped from 6.3 (1 hour) to 3.2 (15 days) to 2.6 (32 days). Apparently the amino function of the glycine was condensed with carbonyl, liberating hydrochloric acid.

**Inhibition of the Browning Reactions.**—The effect of sodium bisulfite in delaying the browning reactions of DPF with glycine is shown in Fig. 2. The addition of quantities of bisulfite equivalent to DPF on a molar basis still did not stop the browning reactions. This result was to be expected, since the reaction of bisulfite with carbonyl groups comes to an equilibrium in aqueous solution. In the presence of 10 moles of sodium bisulfite per mole of DPF, *p*H 5, no increase in optical density could be measured at 4000 Å. after 30 days at 25°.

The browning reactions of DPF with glycine were completely inhibited by adding a particle (*ca.* 10 mg.) of sodium borohydride to the aqueous solution (*p*H 8.5). No color developed after 30 days at 25°. Reduction of the carbonyl group of the fructose derivative[36] prevented enolization and the subsequent dehydration and degradation reactions which accompany browning.

The mention of trade names in this article does not imply endorsement of the products or of the manufacturers.

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