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

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

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

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

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

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

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

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

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

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

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

Introduction

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

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

Recent findings have shown the rearrangement

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

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

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

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

[6] F. Weygand, *ibid.*, **73**, 1259 (1940).

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

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

to be general for many different aldosylamines.[9–11] Examples are also known in which unsubstituted[12] and N-substituted[11,13] D-fructosylamines may rearrange to the corresponding D-glucosamine derivatives.

In previous studies from this Laboratory,[14] the conversion of GPT to DTF was shown probably to be subject to generalized acid–base catalysis. A method also was described for the determination of DTF by titration with 2,6-dichloroindophenol.

Experimental

Typical Reaction Procedure.—A methanolic solution of GPT (0.194 M) was diluted with methanolic solutions of the organic acid (0.25 N) and its potassium salt (0.25 M). The final reaction solution was 0.097 M in GPT, 0.05 N in organic acid and 0.05 N in the potassium salt. A 2.0-ml. aliquot of this reaction mixture was pipetted into a Pyrex tube (8, 10 or 12 mm.), which was then stoppered.[15] The tube was then placed in an ice–water-bath for 10 to 15 minutes, sealed, and the sealed tube placed in a boiling water-bath for a specified time; after the reaction period, it was immediately immersed in an ice–water-bath for at least 5 minutes. The tube then was opened and the contents made up to a definite volume with water. A 1.0-ml. portion of the diluted solution then was titrated under standard conditions with 2,6-dichloroindophenol (Tillmann reagent).[14]

On occasion when the potassium salt was not sufficiently soluble in methanol, 0.10 millimole of the solid salt would be introduced directly into the tube. In these experiments in which DTF was used as the reactant, 52 mg. (0.194 millimole) of solid DTF would also be introduced directly into the tube. In such instances, the sealed tubes were removed within 30 seconds after being placed in the boiling water-bath, shaken to homogenize the resulting solution, and then replaced in the boiling water-bath.

Preparation of Materials.—N-*p*-Tolyl-D-glucosylamine and 1-deoxy-1-*p*-toluino-D-fructose were prepared as previously described.[14]

Anhydrous methanol was prepared by treating absolute methanol (J. T. Baker reagent grade) with magnesium turnings.[16]

Organic Acids.—The best commercial grades of the organic acids were recrystallized, dried over calcium chloride at room temperature, and finally dried to constant weight *in vacuo* over phosphorus pentoxide at 60° or higher temperature when possible. The dry acid was weighed and diluted to the desired normality with anhydrous methanol. The following exceptions occurred.

Solutions (0.25 N) of acetic acid, formic acid and trimethylacetic acid were made up by the direct dilution of glacial acetic acid, formic acid (98–100%) and trimethylacetic acid (m.p. 33–35°), respectively, without prior recrystallization or purification.

Potassium salts were made up directly by adding concentrated potassium hydroxide to an ethanolic solution of the acid, the acid being in slight excess. The precipitate was collected and washed with cold ethanol. The potassium salt was then recrystallized from ethanol and dried to constant weight *in vacuo* at 100° over phosphorus pentoxide. When the potassium salt did not precipitate upon mixing the potassium hydroxide and the acid solutions, the solution was taken to dryness and the residue was collected and treated as described above.

Isolation Procedure.—Isolations were carried out under representative conditions. A typical isolation procedure is described in the following.

A reaction mixture of the following composition was prepared in methanol and heated at 100° for 30 min.: 0.05 N benzoic acid, 0.05 M potassium benzoate, 0.097 M GPT. Titration of an aliquot with 2,6-dichloroindophenol indicated a 63% yield, or 312 mg. in the 19 ml. of reaction mixture. After 3 days storage at −20°, flocculent material which appeared was filtered off and washed with ether to yield 174 mg. of white material, melting at 152–153° with decomposition, $[\alpha]^{23}\mathrm{D}$ −24.7° (methanol, c 0.4). Bayne and Holms[17] gave $[\alpha]^{17}\mathrm{D}$ −23.0 → −10.0° (methanol) for DTF.

The dark mother liquor was evaporated *in vacuo* to dryness at room temperature. The dark brown residue was triturated four times with 5 ml. of cold water. Titration of the solution showed that reducing material equivalent to 47 mg. of DTF passed into the cold water. The residue was then triturated five times with 5-ml. portions of cold ether. Much brown colored material passed into the ether wash, but the residue remained light brown in color. When dried to constant weight *in vacuo*, the washed residue weighed 62 mg. and had a flocculent appearance. Recrystallization of 50 mg. of the residue yielded 39 mg. of a flocculent white precipitate, which melted at 153–154° dec., $[\alpha]^{23}\mathrm{D}$ −24.6° (methanol, c 0.4).

The yield of DTF obtained by the above isolation was 223 mg., or 71% of that calculated by titration with 2,6-dichloroindophenol. If the reducing material which passed into the water wash is considered to be DTF, then the yield was 270 mg. or 87% of that calculated by titration.

Color Measurement.—A 1- or 2-ml. aliquot of reaction mixture (previously diluted about tenfold with water) was mixed with 5 ml. of 0.002 N sodium hydroxide. Preliminary work had established this procedure as best for the color measurement.[1] The color of this solution was read in a Klett photoelectric colorimeter fitted with a no. 42 filter (with center of transmission at 420 mμ). The colorimeter was set to read zero against distilled water. The readings varied from 2 to 600 and were corrected for all dilutions. The readings are reported in the Results as Klett units/ml. of original reaction mixtures. Corrected readings of less than 1000 Klett units/ml. are probably without significance, since they are based on original readings of less than 20 Klett units for the diluted aliquot.

Results

In the present work, the conversion of N-*p*-tolyl-D-glucosylamine (GPT) to 1-deoxy-1-*p*-toluino-D-fructose (DTF) was studied in methanol solution at 100° in the presence of hydrogen chloride, a carboxylic acid or a phenol. For the carboxylic acids and phenols, the reaction also was studied in the presence of equimolar amounts of the salts. The yield of DTF was measured by titration of aliquots of the solution with 2,6-dichloroindophenol, as described previously.[14] Color measurements were also made on many of the solutions at 420 mμ and converted to Klett color units/ml. The destruction of DTF was also studied under representative conditions by titrations and color measurements of similar solutions of DTF.

For 0.097 M solutions of GPT in methanol which contained various amounts of hydrogen chloride, no formation of DTF could be shown by titration. For example, with 0.05 N hydrogen chloride at 100° for 30 min., the yield was less than 1%, and extensive coloration occurred (42,800 units).

Under the same conditions in methanol, acetic acid (0.05 N) produced a yield of 13%, and, in the presence of acetic acid and potassium acetate (0.05 M), DTF was formed to the extent of 30%. The results of a study of the effect of time, acetic acid concentration and potassium acetate concentration are given in Fig. 1.

In the presence of potassium acetate, acetic acid produced yields of DTF as high as 65 to 70% (Fig.

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(9) J. E. Hodge and C. E. Rist, This Journal, **75**, 316 (1953).

(10) A. Abrams, P. H. Lowy and H. Borsook, *ibid.*, **77**, 4794 (1955); P. H. Lowy and H. Borsook, *ibid.*, **78**, 3175 (1956).

(11) J. G. Erickson, *ibid.*, **77**, 2839 (1955).

(12) K. Heyns and K. Meinecke, *Chem. Ber.*, **86**, 1453 (1953).

(13) J. F. Carson, This Journal, **77**, 1881, 5957 (1955).

(14) L. Rosen, J. W. Woods and W. Pigman, *Chem. Ber.*, **90**, 1038 (1957).

(15) The concentrations of the solutes are given at room temperature rather than at the reaction temperature (100°).

(16) L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., New York, N. Y., 1941, p. 360.

(17) S. Bayne and W. H. Holms, *J. Chem. Soc.*, 3247 (1952).

1). The optimal conditions were: acetic acid, 0.6 to 1.5 N, and potassium acetate, 0.38 to 0.75 M. No information was obtained concerning the effect of the concentration of GPT, kept constant at 0.097 M in these experiments.

The marked influence of the acid species and of the presence of potassium acetate confirmed the previous results with pyridine[14] and suggested that a study of the effects of a series of organic acids (and their salts) might provide information useful in interpreting the complex effects. For the formation of DTF from GPT, the catalytic action of nine carboxylic acids and several phenols was measured. The standard conditions were: 0.05 N acid alone or in the presence of an equivalent amount of its salt; GPT concentration, 0.097 M in methanol; time, 30 or 120 min. at 100°. Relatively low concentrations of the acid and salt where chosen primarily because of the low solubility of some of the salts.

Although in the earlier work,[14] the validity of the titration procedure was established as indicating true yields, additional confirmation was obtained in the present work. Eight isolations of DTF were made under representative conditions using benzoic, methoxyacetic, trimethylacetic and *p*-aminobenzoic acids, usually with the salts. The yields of crude DTF were 70 to 80% of that calculated from the titrations.

The yields of DTF catalyzed by the series of organic acids and salts are given in Table I. The results are listed in Table I in order of increasing pK values of the acids in water. The highest yields of 60 to 65% were obtained by the use of acids and their salts with pK_a (H_2O) values in the range 4.2 to 5.1.

TABLE I

THE YIELD OF DTF[a] IN METHANOL AT 100° UNDER DIFFERENT CONDITIONS

Acid	pK_a[b]	0.05 N acid, % yield at 30 min.	120 min.	0.05 N acid and 0.05 M K salt, % yield at 30 min.	120 min.
Hydrochloric	ca. −7[d]	0[e]	.....	..	..
Phenoxyacetic	3.12[c]	10(10)[f]	8(5)[f]	32	27
Methoxyacetic	3.53[c]	11	12	41	45
Formic	3.77[b]	9	8	31	43
Benzoic	4.20[b]	16(26)[f]	23(24)[f]	47	64
Acetic	4.76[b]	13	24	30	65
m-Aminobenzoic	4.79[c]	18[g]	40[g]	27	60
p-Aminobenzoic	4.92[c]	18[h]	38[h]	15	42
Trimethylacetic	5.05[b]	13(25)[f]	33(49)[f]	22	54
m-Dimethylaminobenzoic	5.10[c]	18	29	31	60
p-Nitrophenol	7.14	3	10	<1	6
p-Hydroxyacetophenone	8.05	.....	4[i]	..	3[k]
Phenol	9.95	.....	7[j]	<2	<2

[a] From 0.097 M GPT; 26 mg./ml. = 100%. [b] Reference 18, pp. 573, 577, 579, 588, 589. All values are thermodynamically corrected for water at 25° unless marked *c*. [c] Apparent values for water at 25°. Probably do not differ from the true thermodynamic values by more than 0.1 pK unit.[18] [d] R. P. Bell, ref. 20, p. 46. [e] 0% at 10 minutes. [f] Figures in parentheses are yields from 0.194 M GPT with 0.10 N acid. [g] 7 and 30% at 10 and 180 minutes, respectively. [h] 5 and 34% at 10 and 180 minutes, respectively. [i,j,k] 12, 9, 2%, respectively, at 240 minutes.

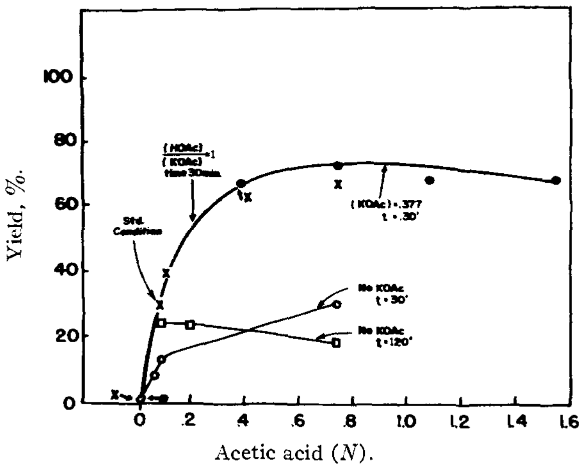

Fig. 1.—Yields of DTF in methanol at 100° with various proportions of acetic acid and potassium acetate.

Since trimethylacetic and *m*-dimethylaminobenzoic acid represent the upper limits of pK_a values for carboxylic acids, phenols were employed to study the effect of still weaker acids. As shown in Table I, the yield of DTF under standard conditions was small for the phenols listed in Table I, always less than 10%. The presence of the potassium salt of the phenol did not improve the yield and usually reduced it.

2,4-Dinitrophenol (pK (H_2O) 4.08)[18] gave a maximum yield of DTF of 26% at 30 min. in the presence of both 0.377 N phenol and its potassium salt. It was not studied under the standard conditions and is not listed in the table. When the salt was omitted, the yield was only 8%. Acetic acid (pK 4.77) under the same conditions gave yields of 66 and 25% (est.), respectively.

Since an increase in reaction time frequently did not improve the yields and often resulted in a decreased yield (Table I and Fig. 1), the existence of other reactions was apparent. These reactions were evaluated in the following ways by studies of several acids under standard conditions. The methods were: (1) loss of DTF as shown by titration of 0.097 M DTF solutions; (2) measurements of the color of these solutions at 420 mμ; (3) measurement of the color at 420 mμ of 0.097 M solutions of GPT. In one set of experiments, both GPT and DTF were present but in half the usual amount; this was done to simulate more closely the actual conditions of reaction, and the results obtained were consistent with the other results.

The results of the studies of the simultaneous and consecutive reactions accompanying DTF formation are given in Tables II and III. The data of Table II show that DTF is destroyed rapidly by the stronger acids and by the salts of weak phenols. The stronger acids produced relatively little color, whereas sodium phenolate (with or without phenol) produced a deep coloration (Table III). The destruction of DTF by the stronger carboxylic acids was decreased by the presence of the salt. This destructive reaction, accompanied by only slight color formation, seems then to be different from

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(18) H. C. Brown, D. H. McDaniel and O. Hafliger in "Determination of Organic Structures by Physical Methods," F. A. Braude and F. C. Nachod, eds., Academic Press, Inc., New York, N. Y., 1955, Chapter 14.

that catalyzed by the salts of the phenols, which produces much color.

TABLE II

CHANGES IN THE AMOUNT OF DTF IN METHANOL AT 100° UNDER VARIOUS CONDITIONS

Acid	Loss,[a] % at 30 min.	Loss,[a] % at 120 min.	Change,[b] % at 30 min.	Change,[b] % at 120 min.
	0.05 *N* acid			
Hydrochloric	98[c]	..	> −98[c]	..
Phenoxyacetic	42	85	−30	−65
Benzoic	9	39	− 5	−25
Trimethylacetic	10	23	0	−10
p-Nitrophenol	..	25	..	..
Phenol	..	3	..	..
	0.05 *N* acid + 0.05 *M* K salt			
Phenoxyacetic	17	52	+14	−32
Benzoic	6	22	+39	+45
Trimethylacetic	9	24	0	− 8
p-Nitrophenol	..	25	..	..
Phenol	87	92	..	..

[a] Initial conditions, 0.097 *M* DTF = 26 mg./ml. = 100%. [b] Initial conditions, 0.0485 *M* DTF + 0.0485 *M* GPT. All % changes are in reference to 13 mg. DTF/ml. = 100%. [c] These values also for 10 minutes.

As shown also by the data in Table III, GPT produced much color by a side reaction not involving DTF. This conclusion is apparent since the color formed from GPT was much greater with the stronger acids than that from DTF. The color formation decreased with increasing *pK* of the acid, and the presence of the salts of the carboxylic acids reduced the color formation markedly. A slight amount of color was produced by the weaker carboxylic acids and the phenols. Since the presence of salts did not decrease the color and sometimes increased the color, this coloration by the weaker acids may have arisen mainly from the base-catalyzed destruction of DTF formed from the GPT.

TABLE III

THE DEVELOPMENT OF COLORATION[a] IN METHANOLIC SOLUTIONS OF GPT AND/OR DTF AT 100° UNDER DIFFERENT CONDITIONS

Acid	0.05 *N* acid 30 min. Klett units/ml.	0.05 *N* acid 120 min. Klett units/ml.	0.05 *N* acid–0.05 *M* K salt 30 min. Klett units/ml.	0.05 *N* acid–0.05 *M* K salt 120 min. Klett units/ml.
	GPT[b]			
Hydrochloric[d]	42800	...	...	...
Phenoxyacetic	30000	48500	12500	15000
Benzoic	7700	18900	1100	5200
Trimethylacetic	2100	8500	1300	7700
p-Hydroxyacetophenone	...	1050	...	2200
Phenol	...	1650	1400	5250
	DTF[b]			
Hydrochloric[e]	1770	...	...	...
Phenoxyacetic	1200	3000	1500	9700
Benzoic	700	2700	700	2800
Trimethylacetic	750	1600	750	3300
Phenol	...	500	28900	40500
	(DTF + GPT)[c]			
Hydrochloric[f]	20800	...	...	...
Phenoxyacetic	15000	23000	6000	15800
Benzoic	4400	12100	1000	3400
Trimethylacetic	1250	5900	850	7000

[a] Measured with a Klett photoelectric colorimeter fitted with a #42 filter. [b] Initial concentration, 0.097 *M* GPT (or DTF). [c] Initial concentration, 0.0485 *M* GPT + 0.0485 *M* DTF. [d,e,f] Klett units at 10 minutes: 32100, 820, 4600, respectively.

The apparently marked effect of the base strength of the anion on the yield of DTF is shown by the results in Fig. 2. Under the standard conditions, the yield of DTF was determined for a weak carboxylic acid (trimethylacetic acid) and also in the presence of equal amounts of its salt or of the salts of two stronger acids. The color was also measured in the solutions. The highest yield (69%) was obtained at 120 min. by the addition of potassium benzoate to the trimethylacetic acid; the color formation was also minimal.

Since in the earlier work, acetic acid in pyridine solvent was found to catalyze the conversion to GPT to DTF, 0.05 *M* pyridine was used in the present work under the standard conditions (at 30 min.) with several acids. Methoxyacetic, benzoic trimethylacetic and hydrochloric acids were used. A slight increase in yield of DTF occurred when both 0.05 *N* acid and 0.05 *M* pyridine were present. The maximum effect was shown for benzoic acid which showed a yield of 16% for the acid alone and of 20% in the presence of pyridine. The effect of the presence of 0.05 *M* pyridine on the color formation in the solution was inappreciable. With and without 0.05 *M* pyridine, considerable color was formed with phenoxyacetic acid (27,000 and 30,000 units); the coloration was much less for the weaker acids (8,000 and 2,000 units).

Experiments also were carried out to determine whether the conversion of GPT to DTF would occur in aqueous solution, under conditions similar to those usually employed for osazone formation. The conditions developed for optimal yields in the present work also were tried using water as a solvent and titrations to indicate yields. With 0.05 *M* GPT in an acetate buffer, no appreciable yield of DTF was produced at 10, 30 and 120 min. at 100°. However, DTF yields ranging from 35 to 45% apparently were produced at 10 and 30 min. for aqueous solutions of the following composition at *p*H 5.0 (100°): glucose, 0.145 *M*; *p*-toluidine, 0.50 *M*; sodium (or potassium) acetate, 0.32 *N*; and acetic acid, 0.51 *N*. Similar yields at 30 min. were obtained when the glucose and a portion of the *p*-toluidine were substituted by molecularly equivalent amounts of GPT. A heating time of 10 to 30 min. seemed optimal.

Discussion

The rearrangement of N-*p*-tolyl-D-glucosylamine (GPT) to 1-deoxy-1-toluino-D-fructose (DTF) in methanol is acid catalyzed, and the catalytic effect on the rearrangement decreases with an increase of the *pK* of the acid. Most phenols appear too weak to be very useful catalysts, except possibly at high concentrations (not studied in the present work). In the one instance in which a comparison with a phenol (2,4-dinitrophenol) and a carboxylic acid (benzoic or acetic acid) of similar *pK* (in water) was possible, the phenol was a less effective catalyst.

Although the pK_a values used for the comparisons

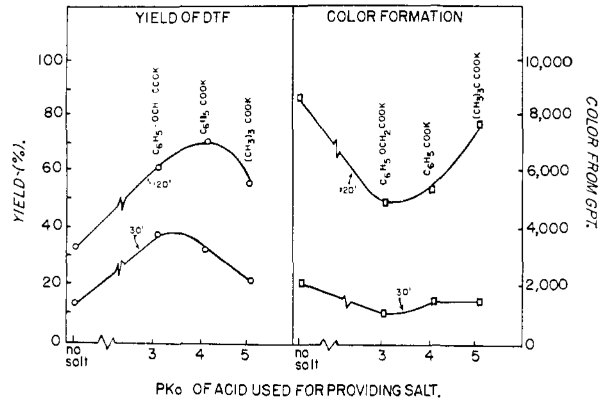

Fig. 2.—Effect of base type with same acid (trimethylacetic acid) on yield of DTF and color formation in methanol at 100°.

were those determined for water, the relative order of acid strengths usually does not change in other solvents,[18–20] but some exceptions to this generalization are known.[18,19] The acid strengths of phenols compared to those of carboxylic acids especially may show marked differences between nonaqueous solvents and water. This effect probably offers the best explanation for the lower yields obtained by the use of 2,4-dinitrophenol (and its salt) in comparison to acetic acid. In 1-butanol, for example, 2,4-dinitrophenol (*pK* 4.77, H_2O) is a much stronger acid than acetic acid (*pK*, 4.08, H_2O).[21] It probably should be compared to a carboxylic acid stronger than acetic acid in water for which increased rates of the destructive reactions would be expected. Picric acid shows a similar greater difference between aqueous and ethanol solutions, relative to acetic acid.[22,23] In contrast to the weakly acidic phenols, 2,4-dinitrophenol behaved like the carboxylic acids in showing an enhanced catalytic effect in the presence of its salt.

In the present work two carboxylic acids, formic and *p*-aminobenzoic, showed some departure from the general pattern. Formic acid gave yields somewhat less than other carboxylic acids of similar *pK* (in water) and *p*-aminobenzoic acid did not show an enhanced effect for its salt. Possibly for the *m*- and *p*-aminobenzoic acids the increased catalytic effect of the free acids may arise from basic catalysis by the amino group.

As indicated in the previous studies of the reaction in pyridine solutions, the conversion of GPT to DTF also seems to be base catalyzed. For acids with optimal *pK* values, the yields were always improved by the presence of the salts, except for *p*-aminobenzoic acid.

The basic catalysis is not clearly distinguishable because of other simultaneous or consecutive reactions. However, a comparison of Figs. 3 and 4 indicates strongly that base catalysis exists, aside from any buffering effect of the salt. Thus, yields increase more in the presence of the salt than can be accounted for by a decrease in the destruction of GPT or DTF (Fig. 4).

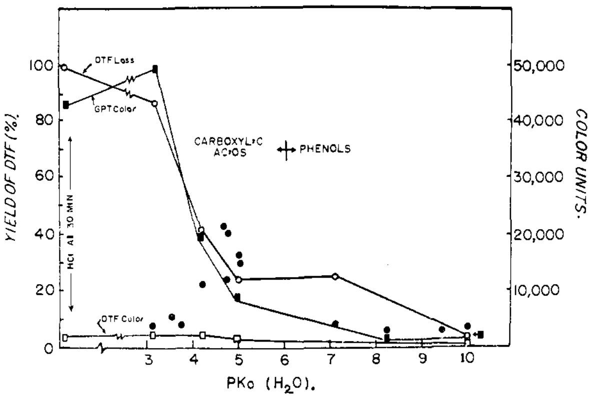

Fig. 3.—Yield of DTF in methanol at 100° at 120 min. in conjunction with side and secondary reactions, for a series of carboxylic acids and phenols (without the salts): ●, yield of DTF (as percentages); ○, DTF loss (as percentage); ■, color formed directly from GPT (as color units); □, color formed from DTF (as color units).

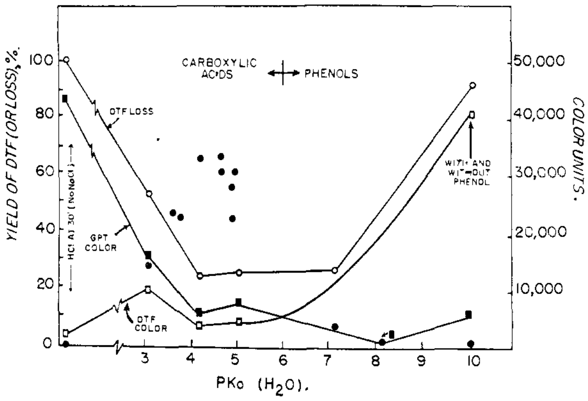

Fig. 4.—Yield of DTF in methanol at 100° at 120 min. in conjunction with side and secondary reactions, for a series of carboxylic acids and phenols and equivalent amounts of the sodium or potassium salts: ●, yield of DTF (as percentages); ○, DTF loss (as percentages); ■, color formed directly from GPT (as color units); □, color formed from DTF (as color units).

As shown in Figs. 3 and 4, the production of maximal yields of DTF seems to be a problem of minimizing the rates of at least three other reactions. These are: (1) an acid-catalyzed destruction of DTF not involving much color formation. (2) A base-catalyzed destruction of DTF giving highly colored solutions. (3) An acid-catalyzed destruction of GPT giving highly colored solutions.

An acid-catalyzed reaction of N-phenyl-D-glucosylamine in methanol to give colored solutions has been studied in this Laboratory[24] and may be related to that from GPT. However, the earlier work was carried out at 30°, whereas the present

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work is at 100°. The color formation from N-phenyl-D-glucosylamine was shown probably to occur for the free base rather than for the cation. The catalytic methonium ion evidently attacks some position in the molecule other than the nitrogen atom.

The acid-catalyzed destruction of DTF produces relatively little color and is thus distinguishable from the GPT coloration reaction. The presence of bases (salts) decreases this reaction rate, most probably by a "buffer effect" on the acid. The retarding effect of salts on the destruction of DTF is especially marked for the acids with pK values of 3 to 4 and seems to be a major factor in obtaining good yields of DTF with these acids.

The difference in the color formation of DTF and GPT by acids also shows that the conversion of GPT to DTF is essentially irreversible under the conditions of the present work.

That base-catalyzed destruction of DTF can occur is indicated by Figs. 3 and 4. Sodium phenolate (with and without phenol) produced an extensive destruction and coloration of DTF. Most likely the color produced from GPT by the phenols (and possibly the weaker carboxylic acids) arose from the DTF formed from the GPT. Thus, in methanol solution, GPT formed only a slight amount of color in 24 hours at 30° in the presence of sodium methylate as concentrated as 0.16 N.

The results shown in Fig. 2 indicate that a very delicate balance of the various reactions exists and that the optimal choice of acid and base is difficult. The choice will also undoubtedly be affected by the basicity of the glycosylamine undergoing the rearrangement.[14,25] For weakly basic glycosylamines, the stronger acids may be required and destructive reactions may predominate. The destructive reactions probably may be minimized by utilizing glycosylamines substituted by benzylidine or other groups.[26]

Weygand[6] was able to produce good yields (about 50%) of DTF from GPT by the use of fusion conditions with hydrogen chloride as a catalyst, whereas no appreciable yields of DTF were obtained by the use of hydrogen chloride under the conditions of the present work. However, in pyridine solution (instead of methanol) yields of DTF as high as 50% were obtained in the earlier work.[14] Evidently under the fusion conditions of Weygand (without solvent) the glycosylamine or free amine is present in high concentration and acts as the base catalyst. As shown in the present work, pyridine in low concentrations (0.05 M) did not exert any catalytic action although it did when present as the solvent. This lack of action may have arisen from a concentration effect or from a change in the acid species.[27]

A similar explanation may be given for the action of "active methylene compounds" in promoting the rearrangement of a number of glycosylamines.[9] Although these compounds are only extremely weak proton donors, as a solvent they may act by forming relatively strong hydrogen bonds at key points of the molecule. However, a different mechanism may also be involved.[28]

Micheel and Schleppinghoff[25] indicated that GPT underwent the Amadori rearrangement in ethanolic hydrogen chloride with yields of 70%. A repetition of this work in our laboratory at reflux temperatures gave a mixture which did not yield a crystalline product and which showed a yield of DTF of less than 2%.[29]

An Amadori rearrangement now appears not to be a step in osazone formation from reducing sugars and hydrazines[30] but cannot be eliminated completely until isotope distribution studies have been carried out. Under conditions for osazone formation (aqueous solutions with acetate buffer), DTF appeared by titration to be formed in yields of 35 to 40% from GPT, and similar yields were obtained from glucose and p-toluidine. These results suggest that improved conditions for osazone formation might be obtained by the use of nonaqueous systems such as methanol or pyridine.

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[27] G. M. Barrow, THIS JOURNAL, **78**, 5802 (1956).

[28] Reference 3, page 179.

[29] Correspondence with Prof. Micheel reveals that the yields reported in Table 1 of ref. 25 are for fusion mixtures and not for alcoholic solutions, although qualitative evidence by infrared analysis and reducing properties indicated some Amadori product present in the alcoholic solutions. We regret that this important work was overlooked in our earlier paper[14] and that his prior study[25] of the effect of aromatic substitution on the ease of the rearrangement was not mentioned.

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