621. N-*Arylglycosylamines.*

By S. Bayne and W. H. Holms.

In the *N*-chlorophenyl- and *N*-tolyl-glucosylamines, and in the *N*-carboxyphenylglucosylamines the *o*-isomers are more stable in air at room temperature than are the *m*- and *p*-isomers. By using reduction of 2 : 6-dichlorophenolindophenol as an index of the transformation it has been shown that, in weakly acidic solutions, the *o*-isomers are less readily converted into the corresponding *iso*glucosamines than are the *m*- and *p*-isomers.

Preparation of the glucosylamine from *p*-toluidine by Sorokin (*J. pr. Chem.*, 1888, **37**, 291) was the first example of the condensation of a sugar with a substituted aniline; and although Irvine and Gilmour (*J.*, 1908, 1429; 1909, 1545) and Irvine and Hynd (*J.*, 1911, 161) prepared a number of compounds of this nature, no comparative study of the

various isomers was possible until Hanaoka (*J. Biochem., Japan*, 1938, **28**, 109) completed the toluidine series and Sannie and Lapin (*Bull. Soc. chim.*, 1948, 892) completed the carboxyaniline series.

Kuhn and Weygand (*Ber.*, 1937, **70**, 769) and Weygand (*Ber.*, 1939, **72**, 1663; 1940, **73**, 1248, 1259) demonstrated the transformation of glycosylamines into *iso*glycosamines by acid and suggested an Amadori rearrangement as its mechanism. *iso*Glycosamines are well-defined crystalline compounds which may be regarded as ketose derivatives, exhibit mutarotation, probably possess a cyclic structure, and, unlike the glycosylamines, reduce methylene-blue and 2 : 6-dichlorophenolindophenol (Kuhn and Birkofer, *Ber.*, 1939, **71**, 621).

During an investigation in this laboratory into the utilisation of aromatic amines as catalysts in the preparation of osazones, glycosylamines were prepared by various methods (Irvine and Gilmour, and Weygand, *locc. cit.*; cf. Kuhn and Ströbele, *Ber.*, 1937, **70**, 773), and some were converted into the isomeric *iso*glycosamines. Many of the glycosylamines and *iso*glycosamines, especially if washed with peroxidised ether, rapidly decomposed to black tarry materials. When standardised methods of preparation and purification were adopted, with precautions to avoid the incorporation of unchanged amine in the crystalline products, certain regularities in behaviour were noticed. The times for formation of *N*-tolyl-D-glucosylamine under standard conditions, as assessed by attainment of homogeneity in the reaction mixture, were *o*- 40, *m*- 25, and *p*- 20 minutes : the glucosylamine is formed more slowly as the substituent methyl group approaches the amino-group. The same regularity was noted for the decomposition : at various rates of decomposition, the rapidity of " browning " was $p > m > o$. This held in a general way also for the glucosylamines from *o*-, *m*-, and *p*-carboxyaniline, and *o*-, *m*-, and *p*-chloroaniline, and the galactosylamines from *o*- and *p*-toluidine. *N*-Phenyl-D-glucosylamine was the least stable of the glucosylamines prepared (cf. Honeyman and Tatchell, *J.*, 1950, 967). The *iso*glucosamines of *o*- and *p*-toluidine, although decomposing much less readily than the corresponding glucosylamines, show the same differential effect of *o*- and *p*-substitution. It thus appears that any substitution in the aromatic ring of certain series of glycosylamines or *iso*glycosamines stabilises the products and that the effect increases with the position of the substituent in the order $p < m < o$.

Unsuccessful attempts were made to measure the rate of decomposition of the glucosylamines by colorimetric estimation of the brown colour produced, according to the method employed by Wolfrom, Cavalieri, and Cavalieri (*J. Amer. Chem. Soc.*, 1947, **69**, 2411) in their studies on the interaction of amino-compounds and sugars. Since an acid environment may lead to hydrolysis (Hanaoka, *loc. cit.*), decomposition, or isomeric rearrangement, depending on such variables as temperature, concentration, type of acid used, and time of reaction, it seems possible that the decomposition of the glycosylamines may proceed *via* aldose residues produced by hydrolysis or *via* the *iso*glycosamines (1) :

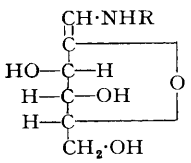
$$\text{Glycosylamine} \longrightarrow \textit{iso}\text{Glycosamine} \longrightarrow \text{Tar} \quad . \quad . \quad . \quad . \quad (1)$$

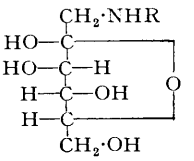
The latter hypothesis is supported by the observation that samples of glycosylamine which have undergone partial decomposition show the reducing properties characteristic of *iso*glycosamine rather than of the free sugar. On the other hand, pure *iso*glycosamines themselves decompose more slowly in acid conditions than the parent glycosylamines and an alternative route (2) for the decomposition process is possible :

$$\text{Glycosylamine} \longrightarrow \text{Unsaturated intermediates} \begin{cases} \nearrow \text{Tar} \\ \rightleftharpoons \textit{iso}\text{Glycosamine} \end{cases} \quad . \quad . \quad . \quad (2)$$

Some support for the second mechanism is afforded by Gottschalk and Partridge's observations (*Nature*, 1950, **165**, 684) on the effect of acid on aliphatic and aromatic glycosylamines. They suggest that (I) may be an intermediate in this decomposition to humin substances and 5-hydroxymethylfurfuraldehyde, being formed by loss of water from (III) which was suggested by Weygand (*Ber.*, 1940, **73**, 1259) as an intermediate in the transformation of glucosylamine to *iso*glucosamine, or from the furanose form (II) of

the *iso*glucosamine. Under mild acid conditions fructofuranose undergoes the same change (Haworth and Jones, *J.*, 1944, 667).


(I)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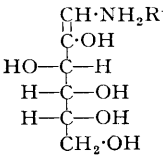

(III)

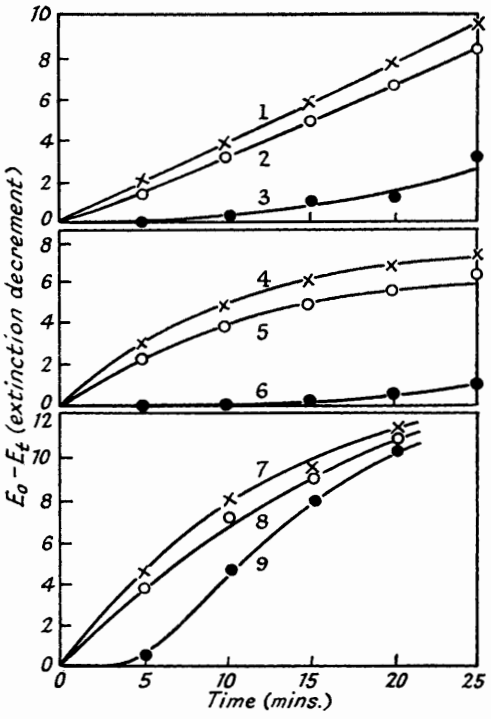
*iso*Glycosamines were prepared by Weygand (*Ber.*, 1940, **73**, 1259) in good yield by heating glycosylamines from several hexoses and pentoses in very dilute hydrochloric or acetic acid. Berger and Lee (*J. Org. Chem.*, 1946, **11**, 76) demonstrated that the *N*-phenyl- and the *N*-*o*-4-xylyl-ribosylamines did not undergo an Amadori rearrangement under these conditions and Berger, Solmssen, Leonard, Wenis, and Lee (*J. Org. Chem.*, 1946, **11**, 91) obtained crystalline ribose by hydrolysing *N*-phenyl-D-ribosylamine in hot dilute acetic acid, removing the amine by steam-distillation or combination with benzaldehyde; however, Kuhn and Birkofer (*loc. cit.*) had noted that certain glycosylamines do not undergo the Amadori rearrangement to *iso*glycosamines and it seems likely that both the structure of the sugar and the nature of the parent amine may determine the type of decomposition. Kuhn *et al.* showed that, unlike the free sugars and the glycosylamines, the *iso*glycosamines reduce 2 : 6-dichlorophenolindophenol (cf. also Berger and Lee, *loc. cit.*). From preliminary experiments it was apparent that at 100° the change was too rapid for convenient measurement by this reagent, and that when the process was carried out in dilute acetic acid the pH changed, altering the colour of the dye. Comparisons were finally made at or near room temperature in an acetate buffer of pH 4·7. This technique, which assumes that the reduction of indophenol parallels the production of *iso*glycosamine, was satisfactory for isomerisations of *N*-tolyl- (curves 1—3) and *N*-chlorophenyl-glucosylamines (curves 4—6), but with *N*-carboxyphenylglucosylamines it gave inconsistent results. The pH of freshly made 1% aqueous solutions of the three *N*-carboxyphenylglucosylamines was 3·7—4·0; thus the irregularities were caused by the ionisation of the free carboxyl groups, catalysing isomerisation to the *iso*glucosamines, the effect varying with the length of time that had elapsed between the preparation and use of the solutions. Preliminary experiments with these glucosylamines showed that other decomposition reactions occurred in alkaline media. The solutions were therefore made up with 0·1N-sodium hydroxide to give ultimate pH values between 5·8 and 6·0; isomerisation was then minimised, alkaline decomposition was avoided, and, when the solutions were used soon after preparation, the carboxyaniline series behaved in the same way as the toluidine and chloroaniline series (curves 7—9).

It is evident that the *o*-isomer, in each series, isomerises more slowly than the *m*- or *p*-isomer—cf. the differing ease of decomposition discussed above. Hanaoka (*loc. cit.*) studied the hydrolysis of these series by mineral acid (see below), and there is an analogous study of the enzyme efficiency (Wertigkeit) of sweet-almond β-glucosidase in the hydrolysis of substituted β-phenylglucosides (Helferich and Phillipp, *Annalen*, 1934, **514**, 228; Helferich and Lutzmann, *ibid.*, 1939, **537**, 11). In the latter substitution by methyl or carboxyl groups increased the rate of hydrolysis in the order $p < m < o$, in direct contradiction to our results for the glucosylamines; glycosides, however, show fundamental chemical differences from glycosylamines and, although the normal forms of certain of the latter are considered to be β-glycopyranosylamines by Butler, Smith, and Stacey (*J.*, 1949, 3371) (cf. also Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) and by Ellis and Honeyman (*Nature*, 1951, **167**, 239) who have, by a different procedure isolated a new isomer of *N*-*p*-tolyl-D-glucosylamine (cf. Irvine and Gilmour, *loc. cit.*), they are not hydrolysed by β-glucosidase (Pigman, *J. Res. Nat. Bur. Stand.*, 1943, **30**, 257).

Hanaoka (*loc. cit.*; *J. Biochem., Japan*, 1940, **31**, 95) followed the hydrolysis of glucosylamine to glucose and free amine (by sulphuric acid in aqueous methyl alcohol) by measuring the rotation of the mixture, assuming that the only optically active substances

present in the solution are glucosylamine as an equilibrium mixture and free glucose; however, as has been shown above, the solution probably contains also the corresponding *iso*glucosamines; in fact Weygand (*Ber.*, 1940, **73**, 1259) has obtained good yields of *iso*glucosamine by employing hydrochloric acid as a catalyst in the Amadori rearrangement. The *iso*glucosamines are in general lævorotatory and the polarimetric method is therefore suspect. Hanaoka's confirmatory method depending on the reduction of alkaline copper solutions does not take cognisance of the fact that any *iso*glucosamine present will effect the reduction even more readily than glucose itself.

Hanaoka showed that in the *N*-tolyl- and *N*-chlorophenyl-D-glucosylamines the rate of "hydrolysis" increases in the order *o*- < *m*- < *p*-, which is directly comparable with our results. In the *N*-carboxyphenyl-D-glucosylamine series, he found that the *o*-derivative was more readily "hydrolysed" than the *p*-derivative. This is at variance with our



N-*Tolyl*-D-*glucosylamines*: 1, p-; 2, m-; 3, o-.

N-*Chlorophenyl*-D-*glucosylamines*: 4, p-; 5, m-; 6, o-.

N-*Carboxyphenyl*-D-*glucosylanmines*: 7, p-; 8, m-; 9, o-.

findings both for decomposition and for isomerisation as measured by the production of indophenol-reducing material. The disagreement may be explicable in terms of the rotational difference between the *iso*glucosamines which are formed under these conditions, and the glucose which was assumed by Hanaoka to be the end-product of the reaction.

Experimental

Amines employed were purified by distillation or by crystallisation from 95% ethanol. M. p.s are corrected. For analysis all compounds were dried *in vacuo* over phosphoric oxide at 60°. Analyses for carbon and hydrogen are by Drs. Weiler and Strauss, Oxford.

N-*Tolyl*-D-*glucosylamines*.—The method of preparation was essentially that of Weygand (*Ber.*, 1939, **72**, 1663), and gave *N*-*o*-tolyl-D-glucosylamine hemihydrate (61·5%), m. p. 95—96°, $[\alpha]_D^{20}$ −79·0° ⟶ −50·0° (*c*, 1·00 in methanol), N-m-*tolyl*-D-*glucosylamine hemihydrate* (74·5%). m. p. 106—107°, $[\alpha]_D^{20}$ −97·5° ⟶ −49·5° (*c*, 1·00 in methanol) (Found: C, 56·2; H, 7·2; N, 5·1. $C_{13}H_{19}O_5N,\frac{1}{2}H_2O$ requires C, 56·1; H, 7·2; N, 5·0%) (Hanaoka, *J. Biochem., Japan*, 1938, **28**, 109, gives m. p. 117°, $[\alpha]_D$ −102·9° ⟶ −50·3°), and *N*-*p*-tolyl-D-glucosylamine hemihydrate (75·8%), m. p. 114—115°, $[\alpha]_D^{20}$ −98·5° ⟶ −46·5° (*c*, 1·00 in methanol).

N-o-Tolyl-D-glucosylamine prepared as above and kept in a desiccator over sulphuric acid did not discolour during 46 days. *N-p*-Tolyl-D-glucosylamine under identical conditions displayed appreciable " browning " after 28 days.

N-*Carboxyphenyl*-D-*glucosylamines*.—Irvine and Gilmour's method (*loc. cit.*) gave *N-o*-carboxyphenyl-D-glucosylamine monohydrate (74·2%), m. p. 131—132° (decomp.), $[\alpha]_D^{18}$ +65·0° ⟶ −9·5° (after 24 hours) (*c*, 0·50 in 50% ethanol).

Sannie and Lapin's method (*loc. cit.*) gave *N-m*-carboxyphenyl-D-glucosylamine monohydrate (77·8%), m. p. 122—123° (decomp.), $[\alpha]_D$ +18·0° ⟶ −41·5° (after 24 hours) (*c*, 0·50 in 50% ethanol), and *N-p*-carboxyphenyl-D-glucoside monohydrate (70·8%), m. p. 132—133° (decomp.), $[\alpha]_D^{18}$ −104° to −105° ⟶ −48·5° (after 24 hours) (*c*, 0·50 in 50% ethanol). Hanaoka (*J. Biochem., Japan*, 1940, **31**, 95) gives m. p. 127°, $[\alpha]_D$ −112·0° ⟶ −51·5° (in methanol), and Dansi (*Farm. Sci. tecn.*, 1947, **2**, 195) m. p. 134—135°, $[\alpha]_D$ −194° ⟶ +30° (in methanol) for the latter.

It was noted by Sannie and Lapin (*loc. cit.*), following the earlier observations of Irvine and Gilmour (*loc. cit.*) on *N-o*-carboxyphenyl-D-glucosylamine, that the mutarotations of the *N*-carboxyphenylglucosylamines depend on the conditions of preparation. In the present investigation the equilibrium values of [α] were not readily obtainable and solutions of these glucosylamines developed a powerful reducing action towards methylene-blue and indophenol after a few hours at room temperature. The complexity of this process appears to explain the variable results reported for the optical rotations of these compounds which have been otherwise clearly defined.

N-*Chlorophenyl*-D-*glucosylamines*.—Irvine and Gilmour's method (*loc. cit.*) gave N-o-*chlorophenyl*-D-*glucosylamine hemihydrate* (70·0%), m. p. 139—140°, $[\alpha]_D^{17}$ −72·0° (*c*, 0·50 in methanol) (Found: C, 48·4; H, 6·1; N, 4·7. $C_{12}H_{16}O_5NCl,\frac{1}{2}H_2O$ requires C, 48·2; H, 5·7; N, 4·7%) (Hanaoka, *J. Biochem., Japan*, 1940, **31**, 95, gives m. p. 137°, $[\alpha]_D$ −50·5° ⟶ +5·0°), N-m-*chlorophenyl*-D-*glucosylamine hemihydrate* (67·1%), m. p. 116—117°, $[\alpha]_D^{17}$ −121·0° (*c*, 0·5 in methanol) (Found: C, 48·5; H, 5·7; N, 5·0%), and N-p-*chlorophenyl*-D-*glucosylamine hemihydrate* (66·7%), m. p. 120—122°, $[\alpha]_D^{17}$ −30° (*c*, 0·5 in methanol) (Found: C, 48·4; H, 5·9; N, 4·7%) (Hanaoka, *loc. cit.*, gives m. p. 126°, $[\alpha]_D$ −40° ⟶ −22·5°). Hanaoka makes no mention of water of crystallisation.

The *N*-chlorophenylglucosylamines did not exhibit measurable mutarotation within a period of 96 hours. After a further 72 hours at room temperature $[\alpha]_D$ had risen by about 2°. The addition of a trace of acid (Hanaoka, *loc. cit.*) initiated a further rise which did not reach an equilibrium value and was accompanied by browning and development of indophenol-reducing properties by the methanolic solution. Kuhn and Birkofer (*loc. cit.*) noted the absence of rotational changes in *N-o*-nitrophenylglycosylamines and the reasons for this were examined by Howard, Kenner, Lythgoe, and Todd (*loc. cit.*).

N-*Phenyl*-D-*glucosylamine*.—When prepared by Weygand's method (*Ber.*, 1939, **72**, 1663), (59%), this had m. p. 134—136°, $[\alpha]_D^{20}$ +10·5° ⟶ −52·2° (*c*, 0·50 in methanol).

N-*Tolylgalactosylamines*.—The method of preparation was essentially that used for the *N*-tolylglucosylamines. The mixture containing *o*-toluidine became homogeneous in 4 minutes and that containing *p*-toluidine in 2 minutes.

N-o-*Tolyl*-D-*galactosylamine hemihydrate* (58·5%) had m. p. 114—116°, $[\alpha]_D^{16}$ −41·0° ⟶ −18·0° (*c*, 1·00 in methanol) (Found: C, 56·3; H, 7·5; N, 5·1. $C_{13}H_{19}O_5N,\frac{1}{2}H_2O$ requires C, 56·1; H, 7·2; N, 5·0%); the *hemihydrate* of the *p*-isomer (72·5%) had m. p. 160—162°, $[\alpha]_D^{16}$ −56·0 ⟶ −23·5° (*c*, 1·00 in methanol) (Found: C, 56·3; H, 7·4; N, 5·4%) (cf. Ellis and Honeyman, *J.*, 1952, 1490).

The *N*-tolylgalactosylamines did not reduce neutral or alkaline 2 : 6-dichlorophenolindophenol but a few minutes after the addition of dilute acetic acid developed reducing properties towards that reagent with decolorisation in either acid or alkaline solution.

N-o- *and* N-p-*Tolyl*-D-iso*glucosamines*.—Weygand's method (*Ber.*, 1940, **73**, 1259) gave o- (62%), m. p. 126—128°, $[\alpha]_D^{17}$ −19·0° ⟶ −4·0° (*c*, 0·50 in methanol) (Found: C, 58·0; H, 7·2; N, 5·8. $C_{13}H_{19}O_5N$ requires C, 58·0; H, 7·1; N, 5·2%) and *p*-tolyl-D-*iso*glucosamine (84%), m. p. 152—154°, $[\alpha]_D^{17}$ −23·0° ⟶ −10·0° (*c*, 0·50 in methanol). Kuhn and Weygand (*loc. cit.*) give m. p. 150—152°, $[\alpha]_D$ −63·8° ⟶ −22·4°. Both showed the characteristic reducing properties described by Kuhn and Birkofer (*loc. cit.*).

Assessment of Glycosylamine and iso*Glucosamine Decomposition*.—A thin layer of pure glycosylamine or *iso*glucosamine on a watch-glass was exposed to an atmosphere saturated with water vapour and acetic acid at about 25°. The time taken for each sample to reach an arbitrarily chosen degree of browning was noted. Simultaneous measurements (see Table)

were carried out on the members of an *o*-, *m*-, *p*-series but no attempt was made to compare the glycosylamines from different amines.

Glucosylamines	*o*	*m*	*p*
Tolyl	40 mins.	12 mins.	8 mins.
Carboxyphenyl ...	75 mins.	20 mins.	15 mins.
Chlorophenyl	20 mins.	9 mins.	6 mins.

Galactosylamines	*o*	*p*
Tolyl	30 mins.	10 mins.
iso*Glucosamines*		
Tolyl	16 hrs.	4 hrs.

Measurement of Rate of Isomerisation to iso*Glucosamine.*—The *iso*glucosamine formed in an acid medium was determined colorimetrically by its reduction of 2 : 6-dichlorophenolindophenol. To a 1% aqueous solution of the glucosylamine (2 ml.) was added 0·01% aqueous 2 : 6-dichlorophenolindophenol (2 ml.). Acetate buffer (pH 4·7; 5 ml.) was added and the gradual disappearance of the red colour at room temperature was measured in a photoelectric colorimeter with an Ilford 621 violet filter, readings being taken at intervals of 5 minutes. Simultaneous observations were carried out on the *N*-tolyl-, *N*-chlorophenyl-, and *N*-carboxyphenylglucosylamines. In the case of the last-named sufficient 0·1N-sodium hydroxide was added to the 1% aqueous solution during its preparation to adjust the pH to 6·0 and prevent autoisomerisation before addition of the acetate buffer and dye.

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