Deoxy-sugars. Part XXVIII. The Stability of Some Substituted Glycosylamines in Aqueous and Non-aqueous Solvents.*

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The chromatographic behaviour of some *N*-substituted D-glycosylamines in aqueous and non-aqueous solvents has been examined. It has been demonstrated that in aqueous solution some hydrolysis of the glycosylamines occurs, and the following equilibrium is set up: *N*-substituted glycosylamine + $H_2O \rightleftharpoons$ (substituted) amine + free sugar. Evidence to support this finding is outlined. The effect of the nature of the amine on the extent of the hydrolysis has been investigated in a preliminary fashion. Comment is made on the effect of this hydrolysis on the changes in optical rotation observed in solutions of these compounds, and the parallel nature of the two changes is noted. Changes in optical rotation of compounds of this class in pyridine are briefly discussed.

As part of a general investigation in this laboratory (cf. Butler, Smith, and Stacey, *J.*, 1949, 3371; Butler, Overend, Smith, and Stacey, *Chem. and Ind.*, 1949, 551; Butler, Laland, Overend, and Stacey, *J.*, 1950, 1433) of the properties of *N*-substituted glycosylamines we have examined their chromatographic behaviour.

On chromatography of aqueous and non-aqueous solutions of 2-deoxy-*N*-*p*-tolyl-D-glucosylamine and -D-galactosylamine, by downward migration on Whatman No. 1 paper

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of the butanol phase of butanol–ethanol–water (5 : 1 : 4) mixture, spots were revealed on development that corresponded in behaviour with the parent 2-deoxyhexoses (*i.e.*, 2-deoxy-D-glucose and 2-deoxy-D-galactose). A similar examination of solutions of *N*-*p*-tolyl-D-galactosylamine showed the presence on the chromatogram of D-galactose (detected by aniline hydrogen phthalate; Partridge, *Nature*, 1949, **164**, 443), together with some of the substituted galactosylamine (detected specifically by the use of *p*-dimethylaminobenzaldehyde; Edward and Waldron, *J.*, 1952, 3631) which had not been hydrolysed. The chromatographic behaviour of these compounds was also examined in anhydrous solvents. No movement of the substances occurred in propylene glycol–toluene (Burton and Zaffaroni, *J. Biol. Chem.*, 1951, **193**, 750; cf. Zaffaroni, Burton, and Keutmann, *Science*, 1950, **111**, 6), benzene–formamide (Burton and Zaffaroni, *loc. cit.*) or anhydrous methanol–heptane (Huelin, *Austral. J. Sci. Res.*, 1952, **5**, *B*, 328). In solvent systems employing methanol, ethanol, propanol, *n*-butanol, and Cellosolve (2-ethoxyethanol) (all anhydrous) either alone or in mixtures, pronounced streaking was encountered. Eventually it was found that the free hexoses could be separated from *N*-arylated hexosylamines by the use of anhydrous Cellosolve–propylene glycol (3 : 1) and, provided that the chromatogram was not overloaded, reasonably discrete spots could be obtained. With this solvent system chromatography of non-aqueous solutions (ethanol and dioxan were used) of the glycosylamines could be effected without significant hydrolysis; only the glycosylamine could be detected on the chromatogram. (In most cases a trace of the free sugar could be detected when the chromatogram was placed in ultraviolet light, but this was attributable to hydrolysis caused by the moisture absorbed during the short periods the chromatograms and materials were unavoidably exposed to the atmosphere.) It was apparent that in the aqueous solvent system initially employed for these experiments the *N*-substituted 2-deoxyhexosylamines had undergone complete hydrolysis, and likewise considerable hydrolysis of *N*-*p*-tolyl-D-galactosylamine had occurred. This was prevented by using a non-aqueous solvent system and in all subsequent chromatographic experiments this was the system adopted.

It is well known that the optical rotation of glycosylamines of this type changes in solution. These rotational changes, particularly those undergone by *N*-substituted D-glucosylamines have been extensively studied (*e.g.*, by Irvine and Gilmour, *J.*, 1908, 1429; Baker, *J.*, 1928, 1583; 1929, 1205; Mitts and Hixon, *J. Amer. Chem. Soc.*, 1944, **66**, 483; Pigman, Cleveland, Couch, and Cleveland, *ibid.*, 1951, **73**, 1976) and in part at least have been attributed to isomerisations, involving interconversions of ring-forms and α- and β-anomers. It is obvious, however, that the marked lability (*i.e.*, hydrolysis) in aqueous solutions of these glycosylamines, as disclosed by our chromatographic experiments, must be borne in mind when considering the changes in optical rotation. Consequently an attempt was made to correlate observations on the extent of the hydrolysis with changes in optical rotation.

The changes in optical rotation of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine under various conditions are shown in Fig. 1. Curves IV and V show the changes in specific rotation of the compound in solution respectively in 50% aqueous ethanol and in water : they are very similar. At equilibrium, chromatography with an anhydrous solvent mixture for irrigation (hence no hydrolysis due to this system) revealed the presence in the solution of non-hydrolysed 2-deoxy-*N*-*p*-tolyl-D-galactosylamine, together with *p*-toluidine and 2-deoxy-D-galactose. That hydrolysis had occurred was confirmed by ethereal extraction of *p*-toluidine from the solution. The presence of unchanged 2-deoxy-*N*-*p*-tolyl-D-galactosylamine on the chromatogram indicated that hydrolysis was not complete. This conclusion was supported by the fact that the final equilibrium value for the optical rotation of the solution did not correspond to the calculated value (based on the measured optical rotation of 2-deoxy-D-galactose in ethanol, and ethanol–water) expected for complete hydrolysis. It is plausible to suggest that the equilibrium shown is set up in aqueous solutions :

$$N\text{-Substituted glycosylamine} + H_2O \rightleftharpoons \text{amine} + \text{free sugar}$$

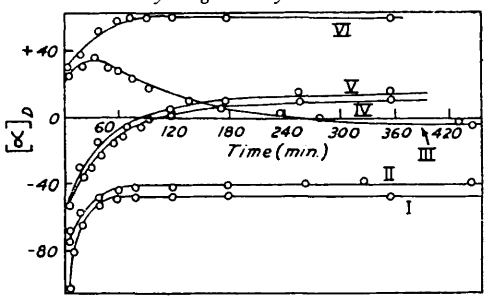
Curve II (Fig. 1) shows the effect of addition of *p*-toluidine (2·5 mols.) on the course of the rotation changes which are normally represented by curve IV : curve II closely parallels

curve I which represents the smaller changes occurring in dry ethanol, when little hydrolysis occurs. Addition of *p*-toluidine favours formation of some of the glycosylamine as would be expected in an equilibrium of the type shown. The reversibility of the changes is also shown by curve III which illustrates the effect of the addition of *p*-toluidine on the course of the mutarotation of 2-deoxy-D-galactose, normally represented by curve VI. Chromatographic examination at the equilibrium state of the solution used to obtain curve III showed the presence in the liquid of some 2-deoxy-*N*-*p*-tolyl-D-galactosylamine, obviously formed by interaction of the sugar and the base.

Comparison of the results depicted in Figs. 1 and 2 (the latter shows results obtained with *N*-*p*-tolyl-D-galactosylamine) indicates the influence of the 2-deoxy-group on the glycosidic centre in the galactose series. Whereas equilibrium of *N*-*p*-tolyl-D-galactosylamine in aqueous solution is attained in approximately 60 hours (Fig. 2, curve IV), the comparable equilibrium of the 2-deoxy-analogue is complete within 5 hours (Fig. 1, curve V).

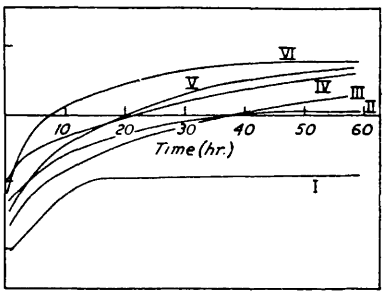
In addition to depicting changes with *N*-*p*-tolyl-D-galactosylamine, Fig. 2 shows the corresponding results obtained with *N*-phenyl-D-galactosylamine. In our experience

FIG. 1. *Optical rotation changes of 2-deoxy-*N*-p-tolyl-*D*-galactosylamine.*



I, 1% *Solution in anhyd.* EtOH.
II, 1% *Solution in* 50% *aq.* EtOH + p-*toluidine* (2·5 *mols.*).
III, 1% *Solution of* 2-*deoxy*-D-*galactose in* 50% *aq.* EtOH + p-*toluidine* (1%).
IV, 1% *Solution in* 50% *aq.* EtOH.
V, 1% *Solution in* H_2O.
VI, 1% *Solution of* 2-*deoxy*-D-*galactose in* 50% *aq.* EtOH.

FIG. 2. *Optical rotational changes of* N-*galactostylamines.*



N-Phenyl-D-galactosylamine.
I, 0·4% *in anhyd.* EtOH.
III, 1·0% *in* 50% *aq.* EtOH.
V, 1·0% *in* H_2O.

N-*p*-Tolyl-D-galactosylamine.
II, 1·0% *in* 80% *aq.* EtOH.
IV, 1·0% *in* H_2O.
VI, 0·5% *in* 50% *aq.* EtOH.

N-*p*-tolyl-D-galactosylamine was not appreciably soluble in anhydrous ethanol and a 0·2% solution could not be obtained. Likewise the 0·4% solution of *N*-phenyl-D-galactosylamine in this solvent was obtained only with much difficulty, but dissolution of these compounds occurred more readily if traces of water were added to the ethanol. The different amine moieties did not materially affect the overall rates of equilibration, although the initial rate of change of the derivative of *p*-toluidine was greater than for the aniline derivative (Fig. 2, curves IV and V respectively). An attempt to determine the effect of basic strength of the amine on the changes was not very successful. In addition to the derivatives of D-galactose with aniline (pK $5{\cdot}3 \times 10^{-10}$) and *p*-toluidine (p$K$ $1{\cdot}5 \times 10^{-9}$), a derivative was made from this sugar and *p*-nitroaniline (pK $1{\cdot}2 \times 10^{-13}$). However, *N*-*p*-nitrophenyl-D-galactosylamine was practically insoluble in water and ethanol. In a saturated aqueous solution no hydrolysis could be detected chromatographically. The substance dissolved in pyridine to give a solution of constant specific rotation. Apparently the weakly basic amines form more stable *N*-glycosides. It is interesting that the absence of hydrolysis is accompanied by a corresponding absence of rotation changes (*i.e.*, no " mutarotation ").

In an equilibrium of the type shown, the hydrolysis of the glycosylamine will result in a pH change in the system. The dependence of the rate of the rotational changes on the pH of the solution is clearly shown in Fig. 3 in which the rotation changes are recorded of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine in various buffered aqueous solutions. Increase in

pH considerably retards the changes, and decrease accelerates them. Comparable results were obtained with the analogous derivative of D-galactose: the rates were much more rapid with the derivative of the 2-deoxy-sugar. Hence, although derivatives of the more basic amines are less stable than those derived from weaker amines, the rate of hydrolysis of the former sugar–base compounds will eventually become very slow owing to the competitive retarding influence of the higher pH developed as hydrolysis proceeds. That an increase in pH occurs in an aqueous solution of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine is evident from the results recorded in Fig. 4. It will be seen that the course of this pH change closely corresponds to that in optical rotation shown in Fig. 1. Fig. 5 shows the corresponding values obtained for pH changes with time of aqueous solutions of *N*-*p*-tolyl- and *N*-phenyl-D-galactosylamine. The measurable increase in the pH of these solutions provides a method of estimating the degree of hydrolysis that has occurred and the extent to which this is responsible for the changes in optical rotation. By comparing the maximum

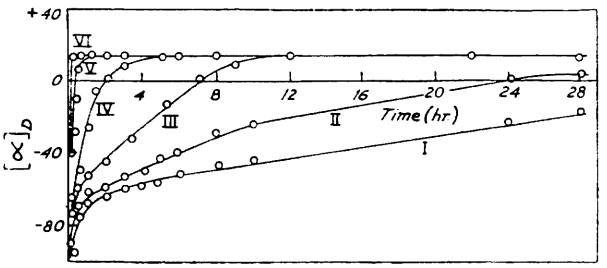


FIG. 3. *Optical rotational changes of* 2-*deoxy*-N-p-*tolyl*-D-*galactosylamine* (1%) *in various buffer solutions.*

Curve	I	II	III	IV	V	VI
pH ...	9·98	9·08	8·59	7·82	7·00	6·00

FIG. 4. *Increase in* pH *of a* 1·0% *aqueous solution of* 2-*deoxy*-N-p-*tolyl*-D-*galactosylamine with time.*

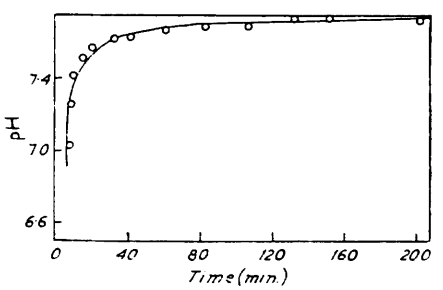
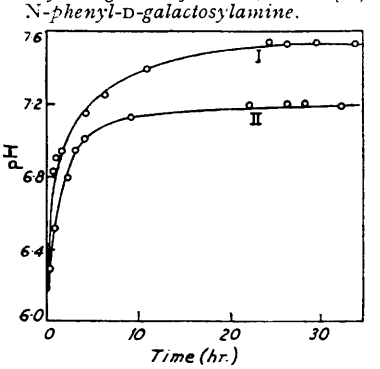


FIG. 5. *Increase in* pH *with time of* 1% *aqueous solutions of* (I) N-p-*tolyl*-D-*galactosylamine, and* (II) N-*phenyl*-D-*galactosylamine.*



pH value attained by a solution of the glycosylamine with that developed by a known concentration of the free base in the same solvent, an estimate of the extent of hydrolysis can be obtained. This method of estimation would not upset equilibrium conditions [previous methods that have been used to determine the extent of the hydrolysis of *N*-substituted D-glucosylamines include extraction of the free base (Mitts and Hixon, *loc. cit.*; Pigman *et al.*, *loc. cit.*), estimations based on the Van Slyke method (Pigman *et al.*, *loc. cit.*), and potentiometric titration of the free base in solution (Mitts and Hixon, *loc. cit.*)]. Since primary amines in solution readily absorb carbon dioxide from the atmosphere (Akashi, *Sci. Papers Inst. Phys. Chem. Res., Tokyo*, 1933, **20**, Nos. 411—413) it is necessary to measure the pH with nitrogen sweeping over the surface of the liquid if reproducible results are required.

Calculations based on results in Figs. 1 and 5, and a calibration graph of the change of pH with concentration of *p*-toluidine in water, gave comparable figures of 87% and 80·5% hydrolysis based on optical rotation and pH measurements respectively for equilibrated solutions of *N*-*p*-tolyl-D-galactosylamine in water. So in this case hydrolysis could account almost entirely for the observed changes in optical rotation.

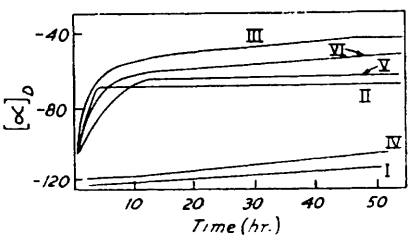
The preceding facts (cf. Barclay, Foster, and Overend, *Chem. and Ind.*, 1953, 462) emphasize the care needed in attributing the changes in optical rotation of solutions of *N*-substituted glycosylamines to true mutarotational phenomena (cf. Irvine and Gilmour, *J.*, 1908, 1429; Irvine and McNicoll, *J.*, 1910, 1449; Kuhn and Dansi, *Ber.*, 1936, **69**, 1745; Weygand, *Ber.*, 1939, **72**, 1663) without prior ascertainment of the effect on the compounds of water and pH changes.

The present results confirm the behaviour of glycosylamines described by previous authors (cf. Mitts and Hixon *loc. cit.*; Pigman *et al.*, *loc. cit.*) and extend the range of generalisations to include derivatives of 2-deoxy-sugars.

As there are conflicting reports on the changes in optical rotation occurring in solutions of glycosylamines in pyridine, we have re-examined the optical rotational behaviour of some derivatives of this type in this solvent. Since we considered that the presence of traces of moisture might account for the differences, great care was taken to exclude moisture, in the compounds and in the solvent and during the experiment. It was then found that neither *N*-phenyl-D-galactosylamine nor *N*-*p*-tolyl-D-galactosylamine underwent any

FIG. 6. *Optical rotational changes of* N-*galactosylamines.*

N-*p*-Tolyl-D-galactosylamine.
I, 1·0% *in dry pyridine.*
II, 1·0% *in dry pyridine* (+5% *of water*).
III, 1·0% *in dry pyridine* (+10% *of water*).
N-Phenyl-D-galactosylamine
IV, 1·0% *in dry pyridine.*
V, 1·0% *in dry pyridine* (+5% *of water*).
VI, 1·0% *in dry pyridine* (+10% *of water*).



significant change in optical rotation during 40 hours (see Fig. 6) (cf. Butler, Laland, Overend, and Stacey, *J.*, 1950, 1433; Ellis and Honeyman, *J.*, 1952, 1490, for observations on these compounds in pyridine solution). Addition of water resulted, however, in considerable changes in optical rotation (Fig. 6, curves II, III, V, and VI).

EXPERIMENTAL

*2-Deoxy-*N-p-*tolyl-*D-*glucosylamine.*—2-Deoxy-D-glucose (0·6 g.) and *p*-toluidine (0·6 g.) were heated together at 95° for 1 hr. After cooling, the solid melt was extracted with ether to remove excess of *p*-toluidine, and the residue recrystallised from a large volume of absolute ethanol. 2-Deoxy-*N*-*p*-tolyl-D-glucosylamine (0·65 g., 69%) was obtained as colourless needles, m. p. 192° (Found: C, 61·75; H, 7·5; N, 5·7. Calc. for $C_{13}H_{19}O_4N$: C, 61·7; H, 7·5; N, 5·5%). Kuhn and Dansi, (*Ber.*, 1936, **69**, 1745) report m. p. 192° for this compound.

2-Deoxy-*N*-*p*-tolyl-D-galactosylamine, prepared similarly (77% yield) from 2-deoxy-D-galactose, had m. p. 143° (from ethanol) and $[\alpha]_D^{20}$ − 44° (equil.) (*c*, 1·0 in EtOH) (Found: C, 61·7; H, 7·5; N, 5·7%).

N-Aryl Derivatives of D-*Galactosylamine.*—(a) *N-Phenyl derivative.* D-Galactose (2·5 g.), aniline (2 g.), and water (0·75 ml.) were heated at 80° for 15 min. and then stirred with absolute ethanol (5 ml.). The solid that separated was recrystallised from ethanol and afforded pure *N*-phenyl-D-galactosylamine (2·7 g., 76%), m. p. 149° (decomp.), $[\alpha]_D^{18}$ − 118° (*c*, 1·0 in dry pyridine) (Found: C, 56·8; H, 6·7; N, 5·5. Calc. for $C_{12}H_{17}O_5N$: C, 56·5; H, 6·7; N, 5·5%). Weygand (*Ber.*, 1939, **72**, 1663) reports m. p. 144°.

(b) N-p-*Tolyl derivative.* D-Galactose (2 g.), *p*-toluidine (1·6 g.), and water (0·6 ml.) were mixed together at 80°. After 10 min. the mixture was homogeneous: heating was continued for a further 15 min. and then the product (2·8 g., 85%) was isolated as described above. It had m. p. 154—156° (decomp.) and $[\alpha]_D^{18}$ − 122° (*c*, 1·0 in dry pyridine) (Found: C, 57·9; H, 7·0; N, 5·2. Calc. for $C_{13}H_{19}O_5N$: C, 58·0; H, 7·1; N, 5·2%). Weygand (*loc. cit.*) reports m. p. 154—155°.

(c) N-p-*Nitrophenyl derivative.* D-Galactose (2 g.) and *p*-nitroaniline (2 g.) were dissolved in methanol (40 ml.), and concentrated hydrochloric acid (0·08 ml.) was added. The solution was heated under reflux for 2 hr. and the crystalline product was filtered off and washed with ether. *N*-*p*-Nitrophenyl-D-galactosylamine (2·5 g., 75%) was obtained as yellow needles, which after recrystallisation from 80% aqueous ethanol had m. p. 218—219° (decomp.) and $[\alpha]_D^{20}$ − 209° (*c*, 1·0 in pyridine) (Found: C, 48·3; H, 5·2; N, 9·35. Calc. for $C_{12}H_{16}O_7N_2$: C, 48·0;

H, 5·4; N, 9·3%). Frèrejacque (*Compt. rend.*, **1938**, **207**, 638) reports m. p. 203° and $[\alpha]_D^{20}$ −202° (in pyridine).

Chromatography Experiments with the N-Glycosides.—Downward migration on Whatman No. 1 filter paper was used throughout. Solutions of the *N*-glycosides were as follows: (*a*) Saturated solution of 2-deoxy-*N*-*p*-tolyl-D-glucosylamine in (i) anhydrous ethanol, (ii) aqueous ethanol, (iii) water, and (iv) anhydrous dioxan; (*b*) 1% solutions of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine in (i) anhydrous ethanol, (ii) aqueous ethanol, and (iii) water; (*c*) a saturated solution of *N*-*p*-tolyl-D-galactosylamine in absolute ethanol and 1% solutions of this compound in aqueous ethanol and in water.

Free reducing sugars and their *N*-glycosides were detected on chromatograms by spraying with aniline hydrogen phthalate reagent and developing at 100° for 3 min. The *N*-glycosides and the free amines were detected specifically by spraying the chromatograms with a solution of *p*-dimethylaminobenzaldehyde [1% in ethanol, diluted (4 : 1) with concentrated hydrochloric acid before use] and development at 80° for 1 min. With this reagent the *N*-glycosides and amines gave a bright yellow complex, whereas the 2-deoxy-D-hexoses gave a grey colour. The normal hexoses were not revealed by this reagent.

(1) *Experiments in aqueous solvents.* Chromatography of the *N*-glycosides of the 2-deoxyhexoses, with as solvent the butanol phase of butanol–ethanol–water (5 : 1 : 4, v/v) and in an atmosphere saturated with the aqueous phase of this mixture, gave a single spot on development. In each case this entity behaved identically with the parent 2-deoxyhexose. Chromatography of *N*-*p*-tolyl-D-galactosylamine under the same conditions showed the presence of two substances on the chromatogram. The slower-moving material behaved in identical fashion to D-galactose, whereas the faster-moving spot gave a bright yellow colour with *p*-dimethylaminobenzaldehyde.

(2) *Experiments in non-aqueous solvents.* The chromatograms were irrigated with anhydrous Cellosolve–propylene glycol (3 : 1, v/v) in an atmosphere saturated with the same mixture. With anhydrous solutions the chromatograms revealed the presence of only one material in each case, which gave a yellow colour with *p*-dimethylaminobenzaldehyde. The R_F values differed from those of the free sugars. Occasionally slight traces of spots having R_F values identical with those of the free sugars were detected in ultraviolet light.

Mutarotational Studies on the Substituted Glycosylamines and their 2-Deoxy-analogues.—The solutions listed in Figs. 1—3 and 6 were used for the mutarotational studies. The pyridine used was distilled six times from phosphoric oxide and was stored in a sealed container. Polarimetric observations on dry pyridine solutions were carried out in a sealed polarimeter tube. The 1% solution of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine in anhydrous ethanol was prepared by heating the mixture for 10 min. Likewise it was necessary to heat *N*-phenyl-D-galactosylamine in anhydrous ethanol for 30 min. in order to obtain a 0·4% solution. *N*-*p*-Tolyl-D-galactosylamine was practically insoluble in anhydrous ethanol even warm.

The buffers employed were:

M/15-Na_2HPO_4–M/15-KH_2PO_4 ...	pH 6·0	M/8-Barbituric acid–sodium barbiturate	pH 8·59
,, ,, ...	pH 7·0	M/10-$NaHCO_3$–M/10-Na_2CO_3	pH 9·08
,, ,, ...	pH 7·82	,, ,, ...	pH 9·98

The solutions studied for mutarotation were also examined by filter-paper chromatography with anhydrous Cellosolve–propylene glycol (3 : 1, v/v). Aqueous solutions of the N-glycosides. gave in each case two spots on the chromatogram. The slower-moving spot behaved as the free sugar and the faster-moving spot gave a bright yellow colour with *p*-dimethylaminobenzaldehyde. Non-aqueous solutions showed only the faster-moving spot, although a trace of the slower-moving material was visible in ultraviolet light.

Measurement of pH *Changes.*—A standard Cambridge pH meter was used. The pH changes of 1% solutions in water of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine, *N*-*p*-tolyl-D-galactosylamine, and *N*-phenyl-D-galactosylamine were noted at intervals (see Fig. 4). The increase in pH with increase in concentration of *p*-toluidine in water was also recorded. Values obtained were:

Concn. in H_2O of *p*-toluidine (%)	0·05	0·1	0·2	0·3	0·35	0·4	0·45	0·5
pH	6·82	7·05	7·35	7·54	7·61	7·65	7·70	7·72

Isolation of p-*Toluidine from an Aqueous-ethanolic Solution of* 2-*Deoxy*-N-p-*tolyl*-D-*galactosylamine.*—After a solution of 2-deoxy-*N*-*p*-tolyl-D-galactosylamine in 50% aqueous ethanol (1% solution in 250 ml.) had been allowed to equilibrate (as shown by the specific rotation) it was diluted with water to 500 ml. and extracted with ether (4 × 50 ml.). The ethereal extract

was dried (Na_2SO_4) and evaporated to dryness. The residue was redissolved in ether, and the solution washed with water, dried, and evaporated. The solid residue (96 mg.) had m. p. 40—42° alone or on admixture with *p*-toluidine. It readily afforded a toluene-*p*-sulphonyl derivative, m. p. and mixed m. p. 116—117° (Muller and Weisenger, *Ber.*, 1879, **12**, 1348, report m. p. 117°).

The aqueous layer remaining after the initial ether-extraction was examined chromatographically [Cellosolve–propylene glycol, (3 : 1)] and the presence in solution of 2-deoxy-D-galactose was demonstrated.

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