

57. *Paper-chromatographic Investigation of the Transglycosylation of N-Arylhexosylamines and Arylamines.*

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Rate of transglycosylation of *N*-arylglycosylamines with other amines has been studied by means of paper chromatography. The relative rates of formation of a new *N*-arylglycosylamine (*a*) by transglycosylation and (*b*) from its components, as well as the hydrolysis of the corresponding glycosylamine, confirmed that the former reaction is a true transglycosylation.

WE report here experiments which in our opinion prove unambiguously that exchange of arylamino-groups between an *N*-arylglycosylamine and a different arylamine is a true transglycosylation (cf. ref. 1); hydrolysis and subsequent reglycosylation are of secondary importance, even in the presence of water.

Our technique consisted of measuring (*a*) the composition of transglycosylation mixtures after various reaction times, using dilute homogeneous solutions, (*b*) the rate of formation of the new glycosylamine from its components, and (*c*) the rate of hydrolysis of the original *N*-arylglycosylamine. Thus, rates of transglycosylation, hydrolysis, and subsequent reglycosylation could be compared.

A method of quantitative paper chromatography was developed for determining *N*-arylglycosylamines and free sugars in the presence of each other, with an accuracy greater than 5%.

¹ Bognár and Nánási, *J.*, 1955, 189; *Acta Chim. Acad. Sci. Hung.*, 1957, **12**, 115; Bognár, Nánási, and Nemes-Nánási, *J.*, 1955, 193.

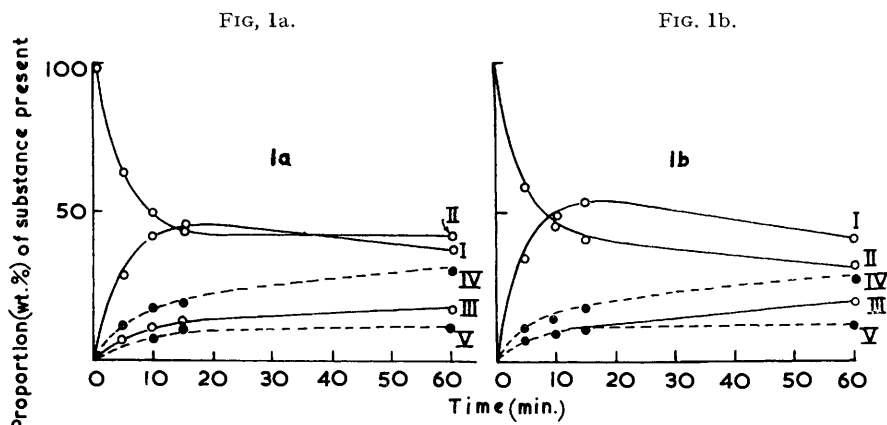


FIG. 1. (a) In presence of HCl. (b) In presence of HCl and pyridine. Rates of (I) formation of N-p-sulphamoylphenyl-D-glucosylamine, (II) removal of N-p-tolyl-D-glucosylamine, and (III) liberation of D-glucose during transglycosylation between N-p-tolyl-D-glucosylamine and p-toluidine. (IV) Rate of hydrolysis of N-p-tolyl-D-glucosylamine. (V) Rate of formation of N-p-sulphamoylphenyl-D-glucosylamine from sulphanilamide and D-glucose.

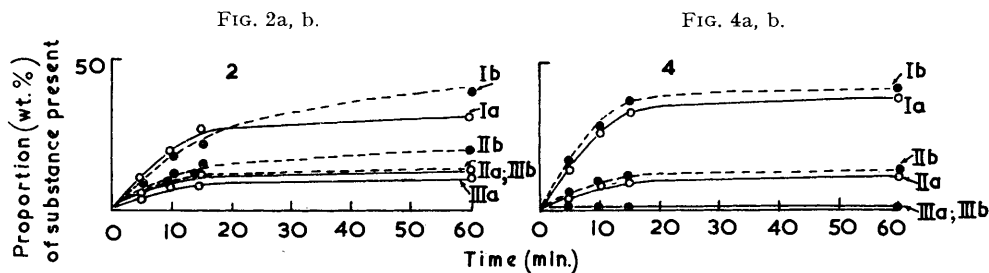


FIG. 2. (a) and (b) as in Fig. 1. Rates of (I) formation of N-p-tolyl-D-glucosylamine from N-p-sulphamoylphenyl-D-glucosylamine and p-toluidine, (II) hydrolysis of N-p-sulphamoylphenyl-D-glucosylamine, and (III) formation of N-p-tolyl-D-glucosylamine from p-toluidine and D-glucose.

FIG. 4. (a) and (b) as in Fig. 1. Rates of (I) formation of N-p-nitrophenyl-D-glucosylamine during transglycosylation between N-p-sulphamoylphenyl-D-glucosylamine and p-nitroaniline, (II) as curve (II) in Fig. 2, and (III) formation of N-p-nitrophenyl-D-glucosylamine from p-nitroaniline and D-glucose.

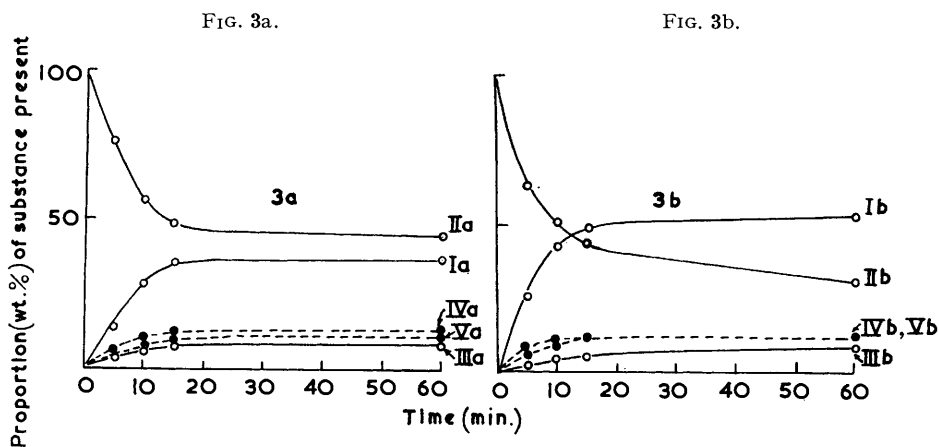


FIG. 3. (a) and (b) as in Fig. 1. Rates of (I) formation of N-p-sulphamoylphenyl-D-glucosylamine, (II) removal of N-p-nitrophenyl-D-glucosylamine, and (III) liberation of D-glucose during transglycosylation between N-p-nitrophenyl-D-glucosylamine and sulphanilamide. (IV) Rate of hydrolysis of N-p-nitrophenyl-D-glucosylamine. (V) As curve (V) in Fig. 1.

In contrast with the preparative experiments, the composition of the reaction medium was chosen to avoid precipitation of either the reagents or the products. Aqueous ethanol 88% was used as the solvent, and hydrochloric acid as catalyst. In parallel experiments a small amount of pyridine was also added, since it was advantageous in transglycosylations.

The results are shown in the Figures. It is apparent that the rate of transglycosylation is, without exception, in all cases considerably higher than that of either hydrolysis or

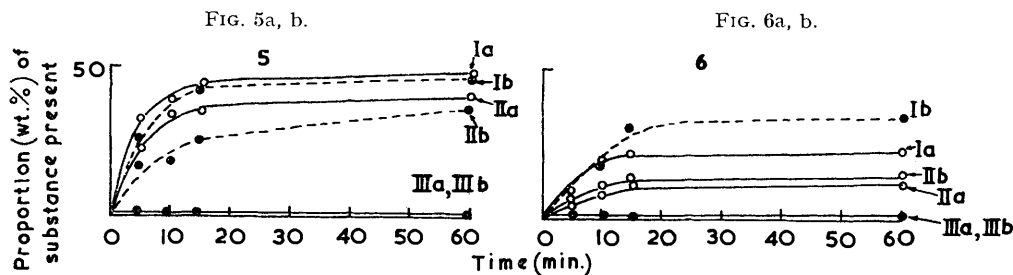


FIG. 5. (a) and (b) as in Fig. 1. Rates of formation of *N*-p-nitrophenyl-D-glucosylamine (I) during transglycosylation between *N*-p-bromophenyl-D-glucosylamine and *p*-nitroaniline and (III) from *p*-nitroaniline and D-glucose. (II) Rate of hydrolysis of *N*-p-bromophenyl-D-glucosylamine.

FIG. 6. (a) and (b) as in Fig. 1. Rates of formation of *N*-p-bromophenyl-D-glucosylamine (I) during transglycosylation between *N*-p-nitrophenyl-D-glucosylamine and *p*-bromoaniline and (III) from *p*-bromoaniline and D-glucose. (II) As curve (IV) of Fig. 3.

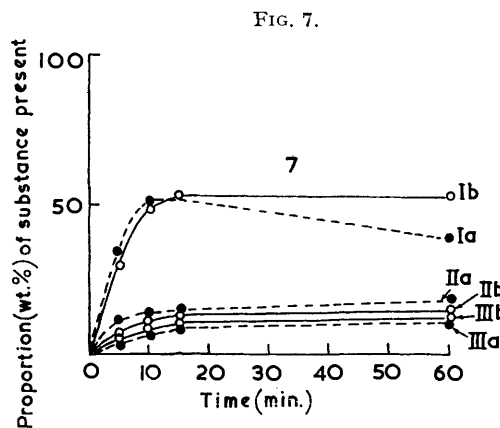


FIG. 7. (a) and (b) as in Fig. 1. Rates of formation of *N*-p-bromophenyl-D-glucosylamine (I) during transglycosylation between *N*-p-sulphamoylphenyl-D-glucosylamine and *p*-bromoaniline and (III) from *p*-bromoaniline and D-glucose. (II) As curve (II) of Fig. 2.

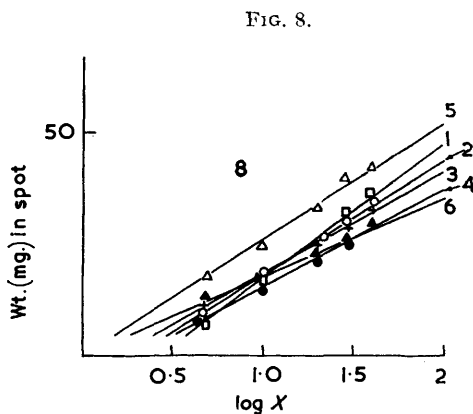


FIG. 8. Calibration curves, for quantitative paper chromatography, of (1) *N*-p-nitrophenyl-, (2) *N*-p-tolyl-, (3) *N*-p-bromophenyl-, and (4) *N*-p-sulphamoylphenyl-D-glucosylamine, (5) *p*-nitroaniline, and (6) D-glucose. X = Wt. (mg.) of material applied.

formation of *N*-arylglucosylamines, even in the dilute, aqueous solutions employed. The rate of the formation of the new *N*-arylglucosylamine from its components is, in general, lower than the rate of hydrolysis even when only the necessary sugar and amine are present as starting materials, in the proper proportions, from the beginning of the reaction. The Figures also prove the reversibility of the transglycosylation² because in each related pair of diagrams (e.g., Figs. 1 and 2) the starting compounds of one are the products of the other.

² Cf. Inoue and Onodera, *J. Agric. Chem. Soc. Japan*, 1953, **27**, 5; *Chem. Abs.*, 1955, **49**, 871.

Experimental.—Composition and sampling of the reaction mixture. A solution 0.04M with respect to each of *N*-aryl-D-glucosylamine, D-glucose, and amine was prepared in ethyl alcohol containing 12% of water. In series (a) hydrochloric acid (0.016 mol.), or in series (b) hydrochloric acid (0.016 mol.) and pyridine (0.025 mol.), were added. The mixture was kept at 40°, and samples (0.1 ml. each) were withdrawn after 5, 10, 15, and 60 min. Reaction was arrested by adding 10% ammonia solution (0.025 ml.) to the samples. Experiments with pure materials showed that under identical conditions no further conversion or hydrolysis of the materials occurred during chromatography.

Analysis by paper chromatography. A portion (0.005 ml.) of the sample was transferred to Schleicher-Schüll No. 2043A paper, and run in butan-1-ol-pyridine-water (6 : 4 : 3). The method of descending chromatography was applied without conditioning of the paper. 10% Aqueous ammonia (5 ml.) was placed in the chamber, and this inhibited further reaction in any direction during chromatography. The running length was 38 cm. in all cases, which required 24 hr. at 20–21°. The area of the spots depended solely on the quantity of the material. The chromatogram papers were allowed to dry at room temperature, sprayed with aniline hydrogen phthalate solution, and dried for 10 min. at 125°. Glycosylamines and free sugars may be detected in this way. The positions of the coloured spots were marked under ultraviolet light.

Quantitative evaluation was by means of calibration curves, prepared by measuring the area of the spots obtained when 5, 10, 20, 30, and 40 µg. of pure *N*-arylglycosylamine and sugar were submitted to chromatography as above. The weight of material in each spot (in mg.), plotted against the logarithm of the quantity of material applied, invariably gave a straight line. Pure materials and components of reaction mixtures of known composition have thus been determined with an error less than 5% (see Fig. 8).

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