änderten Impatiensnektars, und stellt man diese Lösung in den Kühlschrank, so findet man nach 1½–2 Monaten nicht mehr reine Saccharose, sondern ein Gemisch von Fruktose + Glukose + Saccharose + Impatiose. Die Glukose ist in deutlich messbar grösserer Menge vorhanden als die Fruktose, was wiederum auf die postulierte Zusammensetzung des Trisaccharids hinweist. Für die Veränderung der Saccharose kann eine Bakterieninfektion nicht in Frage kommen, denn erstens ist die Lösung steril, und zweitens zeigt eine mit Bakterien oder Pilzen infizierte Lösung stets dehydrierte Produkte. Es können nur Fermente für diesen Umbau verantwortlich gemacht werden, die von der Pflanze mit dem Nektar ausgeschieden wurden.

Da es sich durchweg um Durchlaufchromatogramme handelt, wird statt dem R_F-Wert der R_{Fr}-Wert (auf die Fruktose bezogen) angegeben.

M. ZIMMERMANN

Pflanzenphysiologisches Institut der ETH., Zürich, den 11. August 1952.

Summary

After the injection of different monosaccharides into the phloem of *Impatiens Holstii*, several sugars can be detected in the nectar usually consisting of pure saccharose. Paper chromatography indicates that besides fructose, glucose, saccharose and raffinose a new sugar called "impatiose" is secreted. Hydrolysis suggests that "impatiose" is a trisaccharide composed of two molecules of fructose and one molecule of glucose. The nectar contains enzymes which cause the transformation of sugars.

A New Method for Qualitative and Quantitative Study of Aminoaciduria by Paper Chromatography

The qualitative and quantitative determination of the amino acids excreted in urine has become of importance for the diagnosis of several diseases. Direct paper chromatography of urine samples usually gives poor results. Concentration of many amino acids is low in both normal and pathological human urines, and the presence of urea and salts interfers with the chromatographic separation and the quantitative evaluation.

We have found that preliminary decomposition of urea by urease, followed by desalting of the concentrated urine sample, gives normal, clear chromatograms, without modification of the *Rf* values obtained with pure amino acids solution. This method so increases the sensitivity that traces of amino acids in urine, hitherto undetectable by usual methods, are easily revealed. Such sensitive qualitative analysis is already giving very valuable data to the clinician. For a more accurate evaluation of the amounts of amino acids present, the quantitative determination according to BOISSONNAS[1] is applied and gives results reproducible within a relative limit of ± 10%, which is quite satisfactory for a quantitative clinical study of aminoaciduria.

To 250 cm³ of the urine to be examined, contained in a 300 cm³ round-bottomed neck flask, are added 5 mg of commercial urease (Bios Laboratories Inc., New York). After 24 h at room temperature the flask is placed in a boilling water bath and a glass tube of 5 mm width, connected to a water pump, is introduced through the

[1] R. A. BOISSONNAS, Helv. chim. Acta *33*, 1981 (1950).

neck of the flask so as to reach to about 1 cm above the level of the urine. This procedure allows the concentration of the urine to less than 10 cm³ in 2 to 3 h. The volume of the liquid is completed to 10 cm³ and after filtration the solution is placed in the desalting apparatus described by CONSDEN, GORDON and MARTIN[1]. By the use of an initial current of 1 A the solution is desalted within 4 to 5 h. The solution obtained is eventually neutralized to litmus by a few drops of 1 *M* sodium bicarbonate solution and is now ready for chromatographic separation. The paper chromatographic separation can be effected by any procedure already described for the separation of amino acids from protein hydrolysates[2]. The analysis is effected in triplicate, using 20 mm³ of concentrated desalted urine for each chromatogram. After the chromatographic runs, it is important to dry the paper sheets at temperatures not exceeding 50°C in order to avoid the partial destruction of amino acids which occurs, in the presence of organic solvents, by heating over 50°C. Traces of phenol, which often interfer with the revelation or the chromatography in another direction, are best removed by running 2 chromatographies with ether, which bring the phenol to the front line without displacing the amino acids.

The dry sheets are then heated for 2 h at 100°C and examined under ultraviolet light. The spots are lightly marked with pencil. One sheet is sprayed with a highly sensitive ninhydrine reagent (400 mg of ninhydrine, 90 cm³ of secondary butyl alcohol, 5 cm³ of 1 N aqueous NaOH and 5 cm³ of 2 *N* aqueous acetic acid) and heated for 5 min at 100°C. By comparison with the amino acid spots appearing on this first sheet, the position of the spots on the two other, unrevealed sheets is definitively marked with a pencil. Those two sheets are uniformly sprayed with a 1% methanolic solution of KOH, dried 10 min at 50°C, and the marked spots are cut from the sheets. The amount of amino acids they contain is determined colorimetrically according to BOISSONNAS[3]. The results of this duplicate analysis agree within a relative limit of ± 10%.

R. A. BOISSONNAS and S. LO BIANCO

Department of Organic Chemistry, University of Geneva, August 15, 1952.

Résumé

Une nouvelle méthode pour l'analyse qualitative et quantitative des acides aminés excrétés dans l'urine est décrite. L'urine, concentrée, désalifiée et débarrassée de l'urée qu'elle contient, est soumise à une chromatographie sur papier, et les acides aminés sont déterminés qualitativement ou dosés quantitativement par colorimétrie.

Des traces d'acides aminés, qui ne pouvaient jusqu'ici être mises en évidence par les techniques décrites dans la littérature, peuvent facilement être détectées par cette nouvelle méthode.

[1] R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. *41*, 591 (1947).

[2] R. WILLIAMS and H. M. KIRBY, Science *107*, 481 (1948). – R. A. BOISSONNAS, Helv. chim. Acta *33*, 1966 (1950). – C. E. DENT, Biochem. J. *43*, 169 (1948). – R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. *38*, 224 (1944).

[3] R. A. BOISSONNAS, Helv. chim. Acta *33*, 1981 (1950).