sample gave C, 63·4; H, 3·8 per cent; $C_{26}H_{22}O_{10}$ (II) requires C, 63·2; H, 4·45 per cent; and C_2 $H_{20}O_9$ (IV) requires C, 65·55; H, 4·2 per cent.

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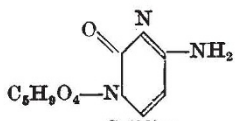
[1] Tollens, B., *Ber. deutsch. chem. Ges.*, **41**, 1788 (1908).
[2] For example, Hanson, S. W. F., Mills, G. T., and Williams, R. T., *Biochem. J.*, **38**, 274 (1944).
[3] Mayer, K., Bloch, H. S., and Chaffee, E., *Fed. Proc.*, **1**, 125 (1942).

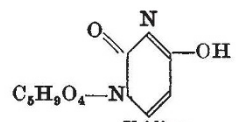
Separation of Pyrimidine Nucleosides by Synthetic Resin Ion-Exchangers

Four nucleosides—guanosine, adenosine, cytidine and uridine—are formed in the hydrolysis of yeast ribonucleic acid by aqueous pyridine, and Bredereck *et al.*[1] have described a method of isolating the nucleosides from the hydrolysate. Although this method is adequate for the production of guanosine and adenosine, we have been unsuccessful in isolating the pyrimidine nucleosides in the pure state and in good yield by this method, and believe this to be due to the method of isolation adopted by Bredereck.

In consequence, a technique has been developed for the separation of cytidine and uridine using a cation-exchange resin.



Cytidine



Uridine

Cytidine would be expected to be retained by a cation-exchange resin due to the basic amino-group ($pK'_b = 9{\cdot}78$)[2]. Uridine, on the other hand, would be expected to have little or no tendency to remain on the resin as the enolic hydroxyl group is acidic ($pK'_a = 9{\cdot}17$)[2]. 'Zeo-Karb 215' was selected for trial because of its strong proton-donating sulphonic acid groups and because of its stability to alkaline solution (*v. infra*). The above expectations were realized when aqueous solutions of cytidine and uridine were separately percolated down columns of 'Zeo-Karb 215'. In the former case, cytidine was afterwards eluted from the column by dilute aqueous ammonia.

A mixture of cytidine and uridine in aqueous solution (0·05 *M* with respect to each nucleoside) was therefore passed down a column of the same resin.

The percolate and aqueous washings were evaporated to dryness, giving crystalline uridine (*c.* 99 per cent recovery), m.p. 162–165° C. The column was eluted with *N*/10 aqueous ammonia and the eluate evaporated to dryness, giving crystalline cytidine (71–78 per cent recovery), m.p. 208–211° C.

An attempt is being made to obtain the pyrimidine nucleosides from deoxyribonucleic acid using the above technique. A fuller account of this work will be published elsewhere.

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March 2.

[1] Bredereck, H., Martini, A., and Richter, F., *Ber. deut. chem. Ges.*, **74** B, 694 (1941).
[2] Levene, P. A., and Simms, H. S., *J. Biol. Chem.*, **65**, 519 (1925).

Isolation and Separation of the Pyrimidine Nucleosides of Yeast Ribosenucleic Acid

Improved methods have been sought for the isolation and preparation of the purine and pyrimidine nucleosides of yeast ribosenucleic acid as a preliminary to the study of the constitution of the nucleic acids of normal and malignant tissues.

Bredereck *et al.*[1] were able to prepare adenosine and guanosine by enzymatic or aqueous pyridine hydrolysis of the acid, and claimed to have separated cytidine and uridine. The yields of these latter were low and no characterization data were given. Gulland and Smith[2] failed to obtain cytidine by Bredereck's method, and the yield of uridine was augmented by deamination of any cytidine present. Isolation of the four nucleosides from the same nucleic acid sample has not hitherto been satisfactorily achieved even from hydrolysates of as much as 100 gm. nucleic acid.

Preliminary experiments showed that cytidine and uridine could be satisfactorily isolated and separated in one operation, not only from artificial mixtures, but also from the pyrimidine nucleoside fraction of the nucleic acid hydrolysate. Cytidine, having a basic amino group ($pK = 4{\cdot}22$)[3], is retained from dilute aqueous solution on percolation through a column of ion-exchange resin 'Zeo-Karb 215', activated by hydrogen ions. Uridine, on the other hand, with an acidic hydroxyl group ($pK = 9{\cdot}17$)[3] is not so retained.

Hydrolysis of 20 gm. yeast ribosenucleic acid (Pharmaco-Chemical Products Co.) with 50 per cent aqueous pyridine, by the method of Bredereck *et al.* (*loc. cit*), gave comparable yields of guanosine (m.p. and mixed m.p. 237–240°) and adenosine (m.p. and mixed m.p. 228–229°). The residue was hydrolysed with 2 per cent sulphuric acid, and the liberated purine bases removed with silver sulphate. Excess silver ions were precipitated as silver sulphide, and sulphate ions as barium sulphate. Percolation of the solution (1 l.) through a column containing the minimum quantity of prepared resin and concentration *in vacuo* of the effluent and 1 l. water washings gave 2·4 gm. uridine, as colourless needles (m.p. and mixed m.p. 165–166°; $\alpha_D^{21} + 10° \pm 2°$; *c*, 1·6245 in water). Elution of the exchanger with 2 per cent aqueous pyridine gave 150 mgm. cytidine (crystallized as sulphate, m.p. and mixed m.p. 224–225° (dec.); $\alpha_D^{21} + 37{\cdot}5 \pm 2°$; *c*, 1·498 in 1 per cent aqueous sulphuric acid).

A volumetric procedure has been developed for the estimation of dilute aqueous solutions of uridine and/or cytidine which may be used to determine the extent of elution or degree of exhaustion of the exchanger.

These experiments are described in detail in a paper submitted and accepted for publication in the *Journal of the Chemical Society* on January 20, 1948.

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[1] Bredereck, H., Martini, A., and Richter, F., *Ber. deut. chem. Ges.*, **74** B, 694 (1941).
[2] Gulland, J. M., and Smith, H., *J. Chem. Soc.*, 338 (1947).
[3] Levene, P. A., and Simms, H. S., *J. Biol. Chem.*, **65**, 519 (1925).