## ADDENDUM

to our paper

## Absolute Configuration of the Spiro Carbon Atom of the Erythrina Alkaloids: Evidence from Optical Rotatory Dispersion

Exper. 19, 108 (1963)

Simultaneously with the appearance of this paper, two communications dealing with the absolute configuration of dihydro- $\beta$ -erythroidine hydrobromide were published. Hanson (Proc. chem. Soc. 1963, 52) derived the complete absolute stereochemistry of this compound from X-ray crystallographic data, while Wenzinger and Boekelheide (Proc. chem. Soc. 1963, 53) presented corroborative chemical evidence. The absolute configuration obtained by these two groups for the spiro carbon atom of dihydro- $\beta$ -erythroidine is the *opposite* of the one derived by us for the corresponding asymmetric center of  $\beta$ -erythroidine and its congeners from a study of their optical rotatory dispersion.

While the reason for this discrepancy is not too clear at present, it appears unlikely that  $\beta$ -erythroidine and its dihydro derivative should have opposite stereochemistry at the spiro carbon atom; it seems possible, therefore, that the interpretation of the optical rotatory dispersion

of heteroannular transoid dienes such as the Erythrina alkaloids is more complex than was apparent at the time the manuscript was prepared. Thus, the proposed rule relating the sign of the Cotton effect to the sense of skewness of the non-planar diene may be vitiated in the present case by the proximity of a hetero atom or the presence of a second chromophore homoconjugated with the diene group  $(\beta, \gamma$ -unsaturated lactone in  $\beta$ -erythroidine, aromatic ring in the other bases). The possibility of an influence of the latter type had been recognized in our paper for the case of dihydro- $\beta$ -erythroidine, but it had not seemed likely that this effect should be strong enough to alter the nature of the chromophore to the point where the rule would become inapplicable. On the basis of the results from X-ray crystallography, this possibility must now be considered.

Until the results of further investigation, now in progress, are available, the possibility of utilizing the sign of the Cotton effect of transoid dienes for elucidation of their stereochemistry must remain sub judice.

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## PRO EXPERIMENTIS

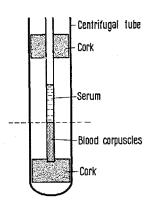
## Preparation of Serum for Paper Electrophoresis from Small Animals

In comparison with free Tiselius electrophoresis only small amounts of serum are needed for paper electrophoresis. Normally 0.01 to 0.005 cm³ of substance is used, depending on the percentage of the material to be analysed. A sufficient amount of blood can be drawn from common laboratory animals by tapping veins or hearts. Animals of rat size can endure a loss of 1–2 cm³ of blood without any damage. But smaller animals are often badly injured or even killed by the loss of such amounts of blood.

If paper electrophoretic methods are used for serological investigation on small animals, the problem is how to get enough blood for serum preparation without hurting the organism, or how to prepare serum from a very small amount of blood.

For this purpose we developed a simple method which gives us enough serum for electrophoresis if only one or two drops of blood are available. The yield is sufficient for two or even three samples. The blood is drawn into a narrow glass or plastic tube of 0.5 to 1 mm diameter. Blood can be obtained by slightly scratching a peripheral vein. The tube is thoroughly closed at one side by a plug of wax or paraffin and then fixed between two corks. Corks and tube are installed in a normal centrifugal tube and centrifugation is performed for 10 min at 10,000 T/min. After separation of the serum from the blood corpuscles, the tube is cut in two between the two phases. Now the serum can easily be brought onto the electrophoretic paper strip.

The method is of special interest for serum investigation of hibernators. Sufficient serum can be obtained for electrophoresis without disturbing the hibernating animals.



Zusammenfassung. 1 bis 2 Tropfen Blut werden in einer Kapillare 10 min bei 10000 U/min zentrifugiert. Die Kapillare wird an der Schichtgrenze zerschnitten. Das Serum kann so leicht auf die Papierstreifen zur Elektrophorese aufgetragen werden.

R. Jaeger

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