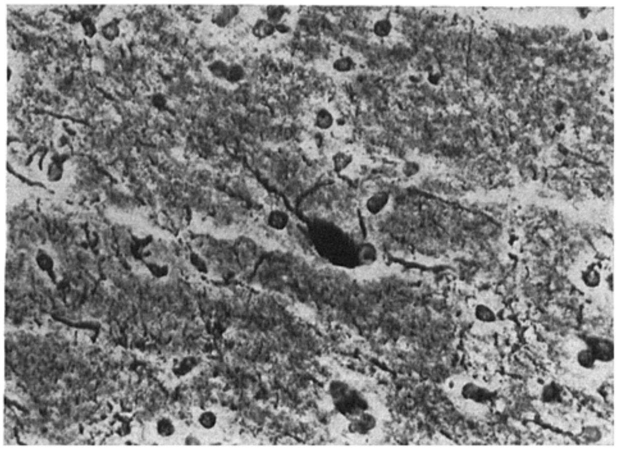

A neurone containing TPN-diaphorase from the frontal cortex of a 44-year old man. This is an enzymatic preparation counter-stained with silver. × 270.

The application of this method to cryostat sections of the brains of rats and humans clearly indicates that the solitary active cells are neurones[4].

Résumé. Les cellules actives isolées du système nerveux central démontrées par la méthode de HESS-PEARSE pour le TPN-H diaphorase, sont des neurones. Ceux-ci sont identifiés par une méthode de coloration argentine que l'on peut pratiquer directement sur les coupes enzymatiques. La recette de cette méthode est donnée.

S. DUCKETT[5] and A. G. E. PEARSE

Pathology Department, Postgraduate Medical School, London (England), December 16, 1963.

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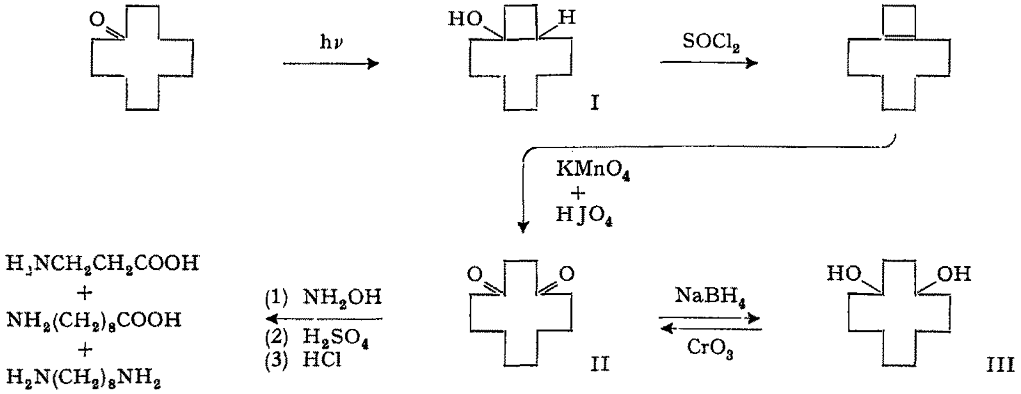
[4] We would like to acknowledge our thanks to Mr. W. BRACKENBURY for the photograph.

[5] Maida Vale Hospital, London.

Photochemical γ-Hydrogen Transfer in Cyclododecanone

A solution of cyclododecanone in *n*-hexane was irradiated at a temperature of 20° with a Hanau 70 w quartz immersion high-pressure mercury lamp in an atmosphere of nitrogen for 2.5 h. Gas and thin-layer chromatographic analysis of the residue showed it to be a mixture of a new substance and of starting cyclododecanone in the ratio 8.5:1.5. Cooling a petroleum ether solution of the mixture caused crystallization of the new substance m.p. 38–40°. *Anal.* calcd. for $C_{12}H_{22}O$: C 79.06; H 12.16; found: C 79.05; H 12.18. ν_{OH}^{KBr} 3360 cm^{-1}, no alcoholic band between 1100–1000 cm^{-1}, weak bands between 1150–1100 cm^{-1}, no UV-absorption.



Structure I was assigned to this compound on the following experimental evidence. Dehydration of I with $SOCl_2$ in pyridine followed by oxidation with $KMnO_4$ + HJO_4[1] gave two fractions. The acidic fraction, owing to the small yield, was not further examined. The neutral fraction, dissolved in petroleum ether, afforded a product II, in white, long needles m.p. 78–80°. *Anal.* calcd. for $C_{12}H_{20}O_2$: C 73.43; H 10.27; found: C 73.25; H 10.36, having the properties of a diketone (reactions with carbonyl reagents, IR absorption ν_{CO}^{KBr} 1700 cm^{-1}). The dioxime of II m.p. 195–197°. *Anal.* calcd. for $C_{12}H_{22}O_2N_2$: C 63.68; H 9.80; found C 63.58; H 9.85, subjected to the Beckmann rearrangement with conc. H_2SO_4, followed by hydrolysis of the dilactams with 6*N* HCl for 48 h furnished β-alanine, 9-aminononanoic acid and 1,8-diaminooctane, identified by high voltage electrophoresis and paper chromatography in comparison with authentic samples[2]. The isolation of the aforementioned compounds permits us to assign to II the structure of cyclododecan-1,4-dione, and therefore to the alcohol I, the structure of bicyclo[8.2.0]dodecan-1-ol.

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[1] R. U. LEMIEUX and E. VON RUDLOFF, Can. J. Chem. *33*, 1701 (1955).

[2] We thank Dr. A. ANASTASI for electrophoretic and Dr. W. BARBIERI for gas chromatographic analysis.

Synthesis of cyclododecan-1,4-dione (II) further confirmed the structures assigned. Cyclododecanol was tosylated and then refluxed with acetic acid-potassium acetate to furnish in excellent yield cyclododecene b.p. 108–110° (12 mm)[3]. Allylic oxidation of cyclododecene with SeO_2 in acetic acid-acetic anhydride[4] afforded an unsaturated diacetate b.p. 160° (1 mm) which was treated in ethereal solution with CrO_3 in dil. H_2SO_4 to eliminate the last traces of selenium.

The diacetate was reduced with Pt/H_2 in acetic acid, and then saponified with alkali to yield cyclododecan-1,4-diol (III) m.p. 145–147°. *Anal.* calcd. for $C_{12}H_{24}O_2$: C 71.95; H 12.08; found: C 71.98; H 12.02, identical to the diol obtained by $NaBH_4$ reduction of II. Oxidation of III gave the diketone II identical by m.p., mixed m.p., IR, thin-layer chromatography to II obtained from I.

Riassunto. Per irradiazione con luce ultravioletta del ciclododecanone in soluzione di *n*.esano si ottiene il biciclo[8.2.0]dodecan-1-olo. La struttura del nuovo composto viene dimostrata.

B. Camerino and B. Patelli

Laboratori Ricerche Farmitalia, Milano (Italy), February 27, 1964.

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Electrophoretic Fractionation of Chloroplast Fragments in a pH Gradient[1]

We have shown that a fraction isolated from sonically ruptured spinach chloroplasts which sediments between 1,000 and 50,000 × *g* exhibits higher Hill activity than intact chloroplasts or fractions sedimented at more than 50,000 × *g*[2]. The highest activity of this fraction resides in a subfraction which can be centrifuged between 20,000 and 50,000 × *g*[3]. The Hill reaction rate and chemical composition of this subfraction, CF_{20-50}, has also been briefly described.

It was reasoned that fragments sedimented on the basis of weight and size might not be chemically uniform, especially since the structure from which they were originally derived, the intact spinach chloroplast, is itself structurally heterogeneous. Thus, it was considered that it might be possible to isolate from the centrifugal fraction, CF_{20-50}, a chemically and structurally pure fraction which might be responsible for the high Hill activity of the parent mixture and would itself exhibit an even higher reaction rate. As an approach to this problem, electrophoretic separation of CF_{20-50} was performed by a modification of the pH and density gradient technique described by Kolin[4].

A simple glass U-tube with side chambers as electrode compartments was used. Electrodes were connected to a Hewlett-Packard model 711A power supply. The U-tube was filled to one third of its height with 50% sucrose-saturated acidic buffer. The subfraction, CF_{20-50}, which had been dialyzed 3 h in the cold against distilled water, was brought to 10 to 25% sucrose saturation; the sucrose concentration depended upon the experiment. The sample was layered above the acid-sucrose buffer in the right-hand arm of the electrophoresis cell. The height of the sample layer was equal to the inside diameter of the tube, as recommended by Kolin. Sucrose-free alkaline buffer was added above the sample layer to fill the tube and adjoining electrode chamber. The opposite arm and its electrode compartment (anode) were filled with sucrose-free acidic buffer; the cathode, then, was in contact with the basic buffer.

The buffer systems used were either Michaelis' universal veronal-acetate buffer[4] or citrate-phosphate buffer prepared according to Gomori[5]. Initially, experiments were performed at a current of about 5 ma. Subsequently, the ionic strength was adjusted with sodium chloride so that the current could be maintained at 20–30 ma, thus permitting good separations in about 5 min. At the end of a run, separated fractions were isolated by means of a 5-ml syringe with a long 18- or 20-gauge needle bent to 90° at the tip to facilitate withdrawal with minimal disturbance of the column.

Figure 1 depicts the results of the electrophoretic fractionations of the chloroplast fraction CF_{20-50}. The sample column in the electrophoretic cell at the beginning of an experiment (Figure 1a) and after 3 min at a current of 5 ma (Figure 1b) is shown.

Both the Michaelis and citrate-phosphate buffers were used with several pH gradients. The citrate-phosphate buffer system providing a gradient of pH 3.0 to 7.0 yielded the sharpest fractionation.

The concentration of suspended material had an effect on the degree of electrophoretic separation. Dilutions of CF_{20-50} with chlorophyll levels of 18, 30, and 60 µg per ml

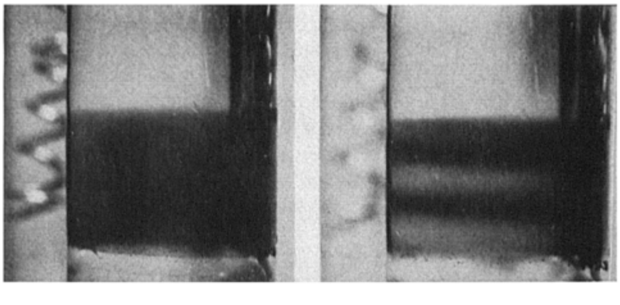

Fig. 1. Electrophoretic separation in Michaelis' buffer of CF_{20-50} from broken spinach chloroplasts. Left – sample column before separation; right – sample column after 3 min, 5 ma, pH 3.0–5.7.

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[1] Supported by Air Force Systems Command, U.S. Air Force, Contract No. AF 33(616)-7255.

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