was obtained when the saponification was carried out in the presence of dimethyl sulfate. The lack of introduction of a new, base stable methoxyl group during this latter treatment ruled out the possibility that reticulol might have a chromone or coumarin skeleton. Further, the derived acid (IIa) formed a 2,4-dinitrophenylhydrazine derivative (IIc). Since neither reticulol nor dimethylreticulol form ketone derivatives, this function must have been generated during saponification; behavior consistent with the presence of the comparatively rare isocoumarin nucleus in reticulol. This was confirmed when ozonization of dimethylreticulol yielded 3,4,5-trimethoxy-phthalic acid[2]. This fact, together with the demonstration of hydrogen bonding between one of the phenolic hydroxyls and the isocoumarin carbonyl, suggests a 6,7,8-trioxygenated aromatic ring with the two free hydroxyls separated by the O-methyl group, as required by the lack of reaction with dichlorodiphenylmethane.

Placement of the remaining C-methyl group at position 3 rather than 4 follows from the production of a diacid on ozonization and also from the ultraviolet spectrum of the 2,4-dinitrophenylhydrazone (IIc), which was more typical of a saturated ketone than of an aldehyde (365 mμ rather than 360 mμ)[3].

Confirmation of this position for the C-methyl group was obtained from the p.m.r. spectrum of reticulol and the derived methyl ester (IIb). The spectrum of reticulol clearly demonstrated the presence of a vinylogously coupled C-methyl group ($\tau = 7.78$; $J = 1$ cps) while that of the methyl ester showed a sharp singlet at 7.82 τ indicative of an uncoupled methyl attached to carbon and another sharp singlet, less intense, at 6.30 τ due to the isolated methylene group.

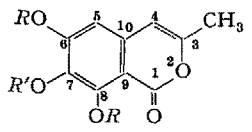
The p.m.r. spectrum of reticulol, measured in deuterated dimethylsulphoxide, provided additional evidence for the hydrogen bonding of one of the hydroxyl groups to the ester carbonyl; one hydroxyl hydrogen appearing at 6.60 τ the other being shifted to -1.13 τ.

Reticulol thus appears to belong to the small, but expanding, class of naturally occurring isocoumarins and, in common with those so far isolated, fits readily into the well known acetate-malonate biogenetic scheme[4].

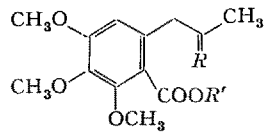
Zusammenfassung. Die Struktur des aus *Streptomyces rubreticulae* isolierten neuen Isocumarins, Reticulol, ist als 3-Methyl-6,8-dihydroxy-7-methoxyisocumarin ermittelt worden.

L. A. Mitscher, W. W. Andres, and W. McCrae

Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River (New York USA), February 24, 1964.



Ia: $R = H$, $R' = CH_3$
Ib: $R = R' = CH_3$
Ic: $R = COCH_3$, $R' = CH_3$
Id: $R = R' = H$



IIa: $R = O$, $R' = H$
IIb: $R = O$, $R' = CH_3$
IIc: $R = N\text{-}NH\text{-}C_6H_3(NO_2)_2$, $R' = H$

[2] O. T. Schmidt and K. Bernauer, Liebigs Ann. *588*, 211 (1954).
[3] J. P. Phillips, J. org. Chem. *27*, 1443 (1962).
[4] R. Bentley, *Annual Review of Biochemistry* (Annual Reviews Inc., Palo Alto, Calif. 1962), vol. 31, p. 589.

The Nature of the Solitary Active Cells of the Central Nervous System

Isolated cells with a high content of $NADPH_2$-tetrazolium reductase (TPN-diaphorase) were reported in the cortex, corpus striatum and pallidum of rats and man by Thomas and Pearse[1,2]. From their shape and from the arrangement of their processes it was deduced that the cells were neurones. Actual proof of this was lacking; however, this communication serves to substantiate the original supposition.

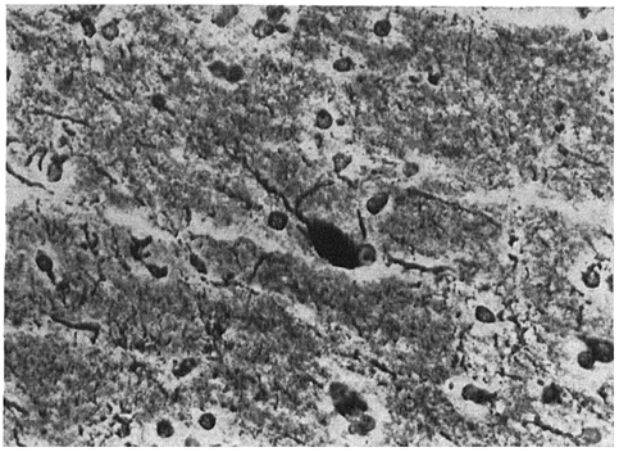
Counter-staining of the enzyme preparation with a silver method (Duckett[3]) can be carried out without loss of the formazan deposit which represents the enzyme activity (Figure).

Method. The method of staining for the TPN diaphorase is given by Thomas and Pearse[1]. The method of counterstaining is carried on from the stage of fixation of the enzymatic preparation in a 15% formol solution. (1) Leave in the 15% formol solution for at least 1 h at room temperature. (2) Wash carefully in three stages of distilled water. (3) Place the section in the silver-ethylamino-oxalate solution for 30 to 60 min at 37°C. (4) Wash quickly in distilled water. (5) Place in a 1% formol solution for 1 min. (6) Wash and mount in glycerine jelly.

The silver-ethylamino-oxalate solution is composed as follows: potassium oxalate 5% solution, 20 cm³; silver nitrate 10% solution, 5 cm³. A white precipitate appears which is dissolved by adding drop by drop a 35% solution of ethylamine, until only a faint deposit is left. Usually about 2 cm³ are needed. Add 10 to 12 drops of absolute alcohol, and make up to 75 cm³ with distilled water. You may add one drop of pyridine to every 5 cm³ of the finished solution.

The section is left untoned and the cells and their nuclei should be a golden yellow, so that the black formazan deposits stand out sharply. The parasite silver deposits are excluded by careful washing in distilled water between steps 1 and 2. As a last resort, if deposits of reduced silver are still troublesome, wash the section at step 2 in a very weak ammonia solution – one drop of ammonia in 10 cm³ of distilled water.

[1] E. Thomas and A. G. E. Pearse, Sonderdruck aus Z. Zellforschung und mikroskopische Anatomie, Abteilung Histochemie *2*, 266 (Springer-Verlag, Berlin 1961).
[2] E. Thomas and A. G. E. Pearse, Acta Neuropathologica, *3*, 238 (1964).
[3] S. Duckett, Acta neuropathol., in press.



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.

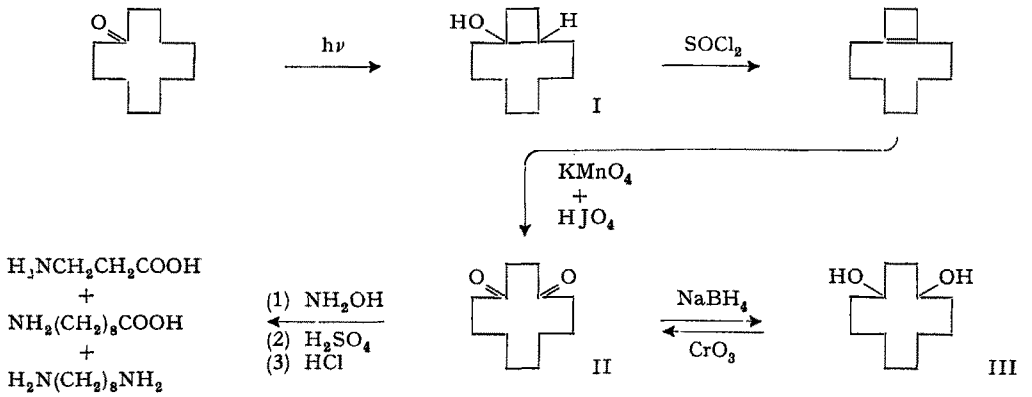
[4] We would like to acknowledge our thanks to Mr. W. BRACKENBURY for the photograph.

[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.



[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.