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The Preparation, Identification and Properties of Chlorophyll Derivatives

A thorough investigation into the identity of chlorophyll derivatives, entitled "Preparation and Properties of 10-Hydroxy Chlorophylls a and b," has been conducted by F. C. Pennington, H. H. Strain, W. A. Svec, and J. J. Katz of Argonne National Laboratory, Argonne, Illinois. This investigation has been based upon several novel techniques that are of wide application not only to pigment investigation but also to the study of many other substances. These methods include modifications of chromatography, and the use of fully-deuterated compounds isolated from fully-deuterated autotropic algae.

The enzymatic oxidation of chlorophyll a and b produced various green substances. The principal oxidation products, which were isolated by chromatography, have been identified as 10-hydroxy chlorophylls. Identification techniques included chemical analysis, chromatography, nuclear magnetic resonance, and infrared spectroscopy. These 10-hydroxy chlorophylls are also shown to be major products of the spontaneous oxidation of the chlorophylls in methanol, the so-called allomerization reaction.

Similar allomerization of the full-deuterated chlorophylls, with H substituted at C-10 by normal exchange, provided the corresponding, fully-deuterated oxidation products, with the −H at C-10 replaced by −OH. These results, verified by n. m. r., provided critical evidence for the molecular structure of the oxidized chlorophylls and strong support for the structure of the methoxy lactones also formed in the allomerization reactions.

In exploratory enzymatic hydroxylation experiments, the formation of the oxidized chlorophylls in leaf material was followed by chromatography of the reaction products in columns of powdered sugar with petroleum ether plus 0.5% n-propanol as the wash liquid. The extent of the reactions was indicated by the diminution of the chlorophyll zones and by the appearance of more-sorbed green substances.

All allomerization experiments were conducted at a chlorophyll concentration of about 0.0067M. Chlorophyll a was dissolved with stirring in 20ml of spectrograde methanol and allowed to stand at room temperature in the dark for three days. The solvent was then evaporated away. The solid residue was chromatographed on powdered sugar using petroleum ether with propanol as a developing solution. Two major fractions were obtained. The one obtained in smallest yield was identical to the enzymatically produced product, which was identified by n. m. r. and infrared spectra as 10-hydroxy chlorophyll a. The second major product had an n. m. r. spectrum consistent with a 10-methoxy lactone structure.

Allomerization of chlorophyll b also yielded two major products. One was the same as the enzymatically produced 10-hydroxy chlorophyll b, the other was consistent with the 10-methoxy lactone formulation.

N. m. r. spectra were recorded on a Varian HA-100 spectrometer. Infrared spectra were recorded on a Beckman IR-7. Absorption spectra in the visible and ultraviolet were recorded on a Cary 14 spectrophotometer. Molecular weights were measured on a Michrolab vapor phase osmometer.

Notes:

1. The report includes a complete discussion of the procedures and results, including sample spectra.
2. Reference: Additional details may be found in *Amer. Chem. Soc.*, 89, 3875, (1967)

(continued overleaf)

3. Inquiries concerning this innovation may be directed to:

Office of Industrial Cooperation
Argonne National Laboratory
9700 South Cass Avenue
Argonne, Illinois 60439
Reference: B68-10409

Source: F. C. Pennington, H. H. Strain, W. A. Svec, J. J. Katz, of the Chemistry Division, (ARG-10205)

Patent status:

Inquiries about obtaining rights for commercial use of this innovation may be made to:

Mr. George H. Lee, Chief
Chicago Patent Group
U.S. Atomic Energy Commission
Chicago Operations Office
9800 South Cass Avenue
Argonne, Illinois 60439