Technical Papers

The Separation of Porphobilinogen and an Ehrlich Negative Precursor of Uroporphyrin[1]

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Hitherto it has been widely accepted that the Ehrlich aldehyde reacting porphobilinogen, excreted in the urine of acute porphyria cases, is the precursor of both uroporphyrin and the nonporphyrin pigment, porphobilin. Waldenström and Vahlquist (*1*) reported that the heating of porphobilinogen-containing urines at pH 4.0 favored uroporphyrin formation, whereas heating at pH 8.0 resulted in a greater formation of porphobilin. They observed the same behavior with porphobilinogen solutions obtained by electrophoresis and regarded as homogeneous entities. Evidence has now been obtained, however, that the Ehrlich-reacting porphobilinogen gives rise only to porphobilin once it has been separated from one or more non-Ehrlich–reacting precursors, which regularly accompany it in the urine of these cases. On the other hand, it has become clear that variable, but often large, fractions of the uroporphyrin in these urines are obtained from non-Ehrlich–reacting precursors. A relatively simple method of separating these substances has now been worked out and is described briefly here.

Urine samples from 8 patients suffering with intermittent acute porphyria were filtered and acidified to pH 5.6–6.0 with acetic acid. A column of Merck's alumina (for chromatographic analysis) was washed with 1% acetic acid until the eluate was acid to litmus. The filtered urine was then run through with gentle suction. Both pigments and chromogens were adsorbed on the column, which was washed first with distilled water and then with 1% acetic acid. The latter contains the porphobilinogen in a relatively concentrated solution. In some cases small amounts of uroporphyrin precursor are also eluted. This solution was brought to pH 6.0 and then irradiated in a Pyrex test tube for 3–5 min with filtered light from an ultraviolet lamp.[2] The irradiation converts any uroporphyrin precursor that may be present to porphyrin, leaving the porphobilinogen unaffected. The solution was barely alkalinized with dilute NaOH, and the free porphyrins were precipitated with $CaCl_2$ and Na_2HPO_4, according to the method of Sveinsson, Rimington, and Barnes (*2*). This final Ehrlich-reacting filtrate was usually free of porphyrin and porphyrin precursors. In certain instances it was necessary to repeat the radiation and $CaHPO_4$ adsorption of uroporphyrin in order to separate the porphobilinogen completely from uroporphyrin precursor.

Solutions of porphobilinogen obtained by the above method, after having been acidified to pH 4.0 and boiled for 30 min, gave rise only to porphobilin, a dark-brown pigment without characteristic absorption spectrum or any porphyrin characteristics.

Electrophoretic studies to be described in detail separately have revealed that porphobilinogen and the uroporphyrin precursor have a single homogeneous zone and cannot be separated on the basis of electrophoretic mobility. The solution of the single zone, however, can then be subjected to the above-described method with complete separation of the two chromogens.

The present observations are believed to explain an incorrect concept that porphobilinogen is a precursor of uroporphyrin as well as porphobilin. Further studies of these precursors are in progress.

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[2] Model BL-2, Black Light Products, Chicago, Ill.

References

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Ultraviolet Absorption Spectra as a Tool for Diagnosing Plant Virus Diseases[1,2]

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Methods developed in this laboratory for the detection of viruses in fruit trees by means of color or staining tests (*1–4*), although useful, are limited in that they are not critical in separating one virus from another. Moreover, these methods are not applicable to herbaceous plants. In a search for more refined methods of virus detection, the ultraviolet absorption spectra of various extracts from virus-diseased and healthy leaves were investigated. After considerable study it was found that acid hydrolysis of leaf tissue, subsequent to the removal of the alcohol-soluble constituents, produced absorption

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