A SIMPLE QUALITATIVE TEST TO DISTINGUISH BETWEEN PROTOPORPHYRIN IX OR ITS ESTERS AND PORPHYRINS CONTAINING NO VINYL GROUP*

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Since a satisfactory procedure of preparing pure protoporphyrin IX dimethyl ester from hemoglobin (1) has been worked out, it is interesting to investigate direct reduction of protoporphyrin IX dimethyl ester into mesoporphyrin IX dimethyl ester. Although these compounds, the melting points of which are too close to be of use for their differentiation, can be distinguished by HCl number (2) or absorption spectra, the simple and rapid qualitative test here described has proved useful in the above investigation.

Protoporphyrin IX dimethyl ester used in this experiment was prepared by a simplified modification of the procedure (1) previously described. A sample of 5 ml. of red blood cells was refluxed with 100 ml. of 10 per cent oxalic acid in acetone for half an hour. The reaction mixture was filtered and washed with a little acetone. The colorless residue was discarded and the solution was then worked up by the procedure outlined in the previous paper. When in place of the 10 per cent oxalic acid solution 5 per cent and 1 per cent solutions of oxalic acid in acetone were tried, the yields of protoporphyrin IX dimethyl ester were 5 mg. and 3 mg. respectively per ml. of red blood cells.

A procedure for preparing protoporphyrin IX dimethyl ester recently described by Grinstein and Camponovo (3) was also tried and modified. A sample of 5 ml. of red blood cells was stirred with 100 ml. of 1 per cent oxalic acid in methanol. The dark solution separated from the residue by centrifuging was shaken with 5 gm. of stannous chloride and mixed with an equal volume of methanol previously saturated with hydrogen chloride gas. The reaction mixture was allowed to stand at room temperature for 1 to 2 hours and was worked up as usual. By this modified method a pure product was obtained without the necessity of chromatographic purification, the yield being 2.8 mg. per ml. of red blood cells.

Mesoporphyrin IX dimethyl ester was also directly prepared from red blood cells. The acetone solution resulting from refluxing 5 ml. of red

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blood cells with 100 ml. of 1 per cent oxalic acid in acetone was distilled under reduced pressure at room temperature to remove the solvent. The residue was mixed with excess water and centrifuged. The dark residue was washed once with water and dried on a steam bath. It was then reduced with a mixture of 0.35 ml. of hydriodic acid (sp. gr. 1.7) and 2.6 ml. of glacial acetic acid according to the procedure of Fischer and Kogl (4). A methanol solution of crude mesoporphyrin thus obtained was mixed with an equal volume of methanol previously saturated with hydrogen chloride and allowed to stand at room temperature for 1½ hours. The ester was extracted as usual and purified by chromatographic separation, the yield being 3 mg. per ml. of red blood cells.

For the qualitative test protoporphyrin IX dimethyl ester and mesoporphyrin IX dimethyl ester were respectively dissolved in chloroform as pink solutions. Then to each tube were added 3 drops of concentrated hydrochloric acid with vigorous shaking. Changes in color were observed in transmitted daylight. The solution containing mesoporphyrin IX ester is purple, while that containing protoporphyrin IX ester shows a green shade. In both tubes dihydrochlorides were formed which showed different colors toward transmitted daylight. In either tube the pink color was restored by addition of excess saturated sodium acetate solution and the test could be repeated. Free protoporphyrin IX gave the same color change as its ester, while free mesoporphyrin and other porphyrins, such as coproporphyrin, containing no vinyl group behaved toward hydrochloric acid just like mesoporphyrin IX dimethyl ester. Therefore this simple test serves as a rapid means of distinguishing between porphyrins of the protoporphyrin type and porphyrins containing no vinyl group. The test is sensitive in a concentration of 10 to 100 γ per ml. of testing solution.

SUMMARY

Protoporphyrin IX dimethyl ester and mesoporphyrin IX dimethyl ester were respectively prepared by modified procedures. A simple color test to distinguish between these two porphyrins is described.

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