The Isolation of a Green Pigment from Yeast

W. J. RUTTER* and BRITA ROLANDER

Medicinska Nobelinstitutet, Biokemiska Avdelningen, Stockholm, Sweden

In connection with attempts to find efficient means of extraction of various oxidative enzymes, yeast cells were subjected to various treatments with non-aqueous solvents. After certain preextraction conditions deep green solutions were obtained. The green pigment has been concentrated and partially purified. A colorless material present as a contaminant these purified green preparations has been crystallized.

The primary observations, together with certain of the chemical properties of the compounds are presented in this communication.

Results and discussion. Fresh bottom yeast kindly furnished by Stockholm's Hamburgerbryggeriet was washed by decantation several times with cold water and finally when the washes were almost colorless, the yeast was collected by centrifugation. The yeast cake was then extracted twice with 2 volumes ethylene glycol at −12°C. Short-time homogenization with a blendor in the cold was useful at this point. 2 volumes of acetone were added, and the very dark brown solution was filtered off at −12°. The next acetone extract was noticeably green, but was usually discarded. Extraction with ethyl ether then gave a deep green or in some cases a yellowish green solution.

If the ethylene glycol preextraction step was omitted, the procedure was much less successful. Preextraction with other agents, for example glycerol, was also less satisfactory.

All subsequent steps were carried out in the dark. The first extract was concentrated to an oil *in vacuo* at 10°C.; the oil was then dissolved in a minimum amount of acetone. A 5 ml aliquot of the green solution (equivalent to a total of 5—10 O.D. units at 650 μ) was then placed on a powdered cellulose column (3.7 × 30 cm). After washing the column with 10 volumes methanol, the green pigment remained as a narrow band at the top, while much of the yellow contaminants migrated down the column. Gradient elution with increasing concentrations of ether in acetone yielded a sharp fraction containing the green compound(s). The green material was concentrated to an oil and dissolved in acetone. Addition of methanol with cooling produced a heavy crop of colorless crystals which were removed by filtration and washed with cold methanol.

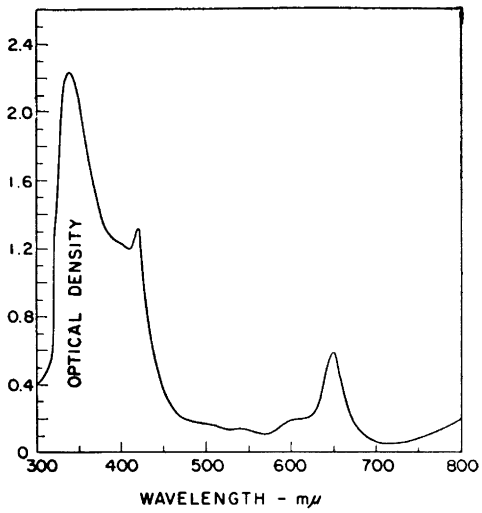

Fig. 1. Absorption spectrum of purified green pigment obtained from yeast.

The remaining deeply green solution was then concentrated and stored as an oil in the dark. The absorption spectrum of the purified green pigment is shown in Fig. 1. The spectrum is similar in form to certain hematin compounds, especially the chlorophylls [1,2]. However, no pyridine hemochromogen could be obtained under the usual conditions. Analysis of the preparation by chemical and spectroscopic means showed the presence of low concentrations of iron, and rather more magnesium. No other metals were detected. Addition of $FeCl_3$ to the green compound produced an immediate conversion to a yellow compound. The green material fluoresces intensely when irradiated with ultraviolet light, but solutions

* Postdoctoral fellow of United States Public Health Service, 1954—55; present address, Division of Biochemistry, University of Illinois, Urbana, Illinois.

are also self-quenching. The colorless crystals did not fluoresce.

The spectrum of the white crystals ($CHCl_3$) in the infra red region showed prominent absorption peak at 2 940 with a shoulder at 2 870 cm^{-1}. Less prominent peks are at 1 725, 1 605, 1 467, 1 380, 1 060, 1 035, 985 and 842 cm^{-1}. The purified green material exhibited the same prominent peak at 2 920 cm^{-1} and the shoulder at 2 860 cm^{-1}, and in addition, the absorption at 1 730 cm^{-1} was very much intensified. There was a great deal of generalized absorption from 900—1 500 cm^{-1}, with two distinct peaks at 1 465 cm^{-1} and 1 385 cm^{-1}. The spectra are not overly informative, but do suggest the possible presence of aldehyde or COOH groups in the green compound, while the white crystals could be a saturated lipid having comparatively few functional groups.

The question whether the green compound is a natural constituent with a role in the physiology of the yeast cell cannot be answered unequivocally. It can be stated that the green compound was consistently obtained from each of five different air-dried brewers yeast preparations in this laboratory. This procedure, however, did not yield a green product from bakers yeast. It is pertinent, however, that Keilin [3] reported a green fraction in initial attempts at the isolation of cytochrome c from brewers yeast in 1931. Efforts to obtain the present green compound by a suitable modification of Keilin's procedure have not proved successful.

There is some evidence which suggests that some of the efficiency of extraction of the green compound is very closely related to the state of the cells. Thus it has not been possible to obtain the green compound by extraction of fresh yeast cells, nor of cells which have been autolyzed even for short periods. It has also not been possible to obtain the green compound from freeze-dried preparations. Suitable experiments indicate that the green color is not due to an artifact of the reagents used.

The actual chemical structure of the green compound and its role in biological systems awaits clarification. It would be particularly interesting to know if present compounds have a relation to any of the physiologically important green compounds known.

Acknowledgment. The authors wish to express appreciation to Professor Theorell for his active interest in this work, and W. J. Rutter wishes to express his thanks for the gracious hospitality extended by Professor Theorell and other members of the Biochemical Department.

1. Lemberg, R. *Hematin Compounds and Bile Pigments*, Interscience Publishers, New York 1949.
2. Rabinowitch, E. I. *Photosynthesis*, Interscience Publishers, New York 1945—1951.
3. Keilin, D. *Proc. Roy. Soc. London* **B 106** (1930) 418.

Received December 19, 1957.

Examination of the Behaviour of Liquid Mixtures in the Critical Region

HANS LUDVIG LORENTZEN and BJØRN BERGSNOV HANSEN

Universitetets Kjemiske Institutt, Blindern-Oslo, Norway

In order to investigate the shape of the co-existence curves close to the critical point, the two systems of partly miscible liquids, triethylamine-water (at the lower critical point) and succinonitrile-ethanol have been examined. The results are shown in Fig. 1, where the co-existence curves are seen to follow the "cubic rule" [1,2]

$$\Delta c = k \cdot \sqrt[3]{\Delta T} \qquad (1)$$

Δc being the composition of the individual phases measured from the critical, and ΔT the temperature distance from the critical.

It will be observed from Fig. 1 that in the case of triethylamine-water the plotted results follow the straight line as close as 1 mdeg to the critical. However, uncertainties regarding the fixation of the critical temperature involve uncertainty as regards the plots from the critical temperature to about $\Delta T = 2$ mdeg. Nevertheless, the visually obtained information about the initiation of the two phases when passing the critical temperatures