Communications to the Editor

U.D.C. 547.97:582.284

Isolation of a Green Fluorescent Substance produced by *Eremothecium ashbyii*

The writer previously studied by paper chromatography components of the mycelium and broth collected at various stages of the culture of *Eremothecium ashbyii* and reached the conclusion that FAD was formed from riboflavin and ATP during the culture[1]. In the same work, a green (G-substance) and a purple (V-substance) fluorescent substances, besides riboflavin, ATP, and FAD, were detected on the chromatogram, especially clearly with the material collected at a comparatively early stages of the culture. The fluorescent substances were further separated by paper chromatography with a large-size filter paper and their Rf values by developments with various solvents and their migration in paper ionophoresis were also investigated. The writer has thereafter succeeded in isolating the green fluorescent substance in crystalline form, with which the present paper deals.

When the development was conducted with benzyl alcohol-water mixture instead of ethanol:butanol:water (15 : 50 : 35), which was used in the previous work, it was found that FAD and the V-substance did not move, whereas the G-substance and riboflavin were separated with Rf values 0.22 and 0.60, respectively. Therefore, the mycelium obtained by 40- to 60-hour culture of *Eremothecium ashbyii* was extracted with water at 80° for 15 minutes and the extract, after concentration, was mixed with ammonium sulfate and extracted with phenol. Ether was added to the phenol extract, the aqueous layer was concentrated, and the residual substance was developed on a column of powdered cellulose with benzyl alcohol or a column of Florisil with pyridine-water. The G-substance thus purified and collected was recrystallized from diluted ethanol to light yellow needles, m.p. 273~274°(decomp.); $[\alpha]_D^{20} = -164°$. *Anal.* Calcd. for $C_{14}H_{18}N_4O_7$: C, 47.20; H, 5.09; N, 15.70. Found: C, 47.48; H, 4.74; N, 15.36.

This substance is readily soluble in water, acetic acid, and pyridine, sparingly

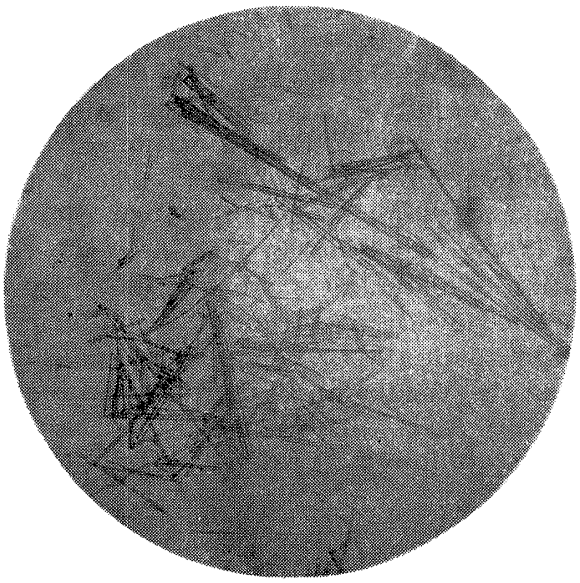

Fig. 1. Crystals of G-Substance

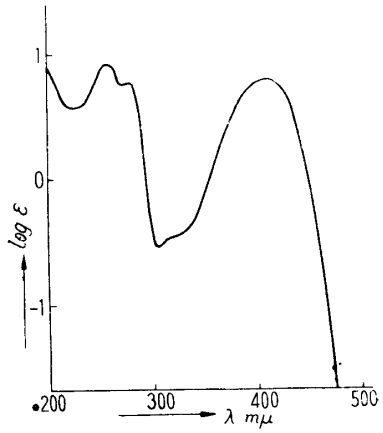

Fig. 2. Ultraviolet Absorption Spectrum of G-Substance

1) T. Masuda: This Bulletin, **3**, 434(1955).

NII-Electronic Library Service

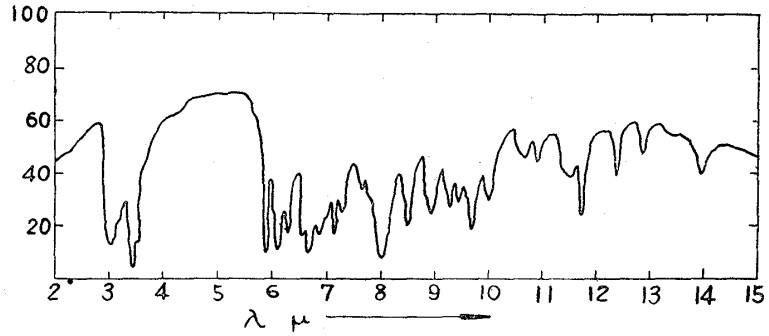

Fig. 3. Infrared Absorption Spectrum of G-Substance (in Nujol)

soluble in ethanol and methanol, insoluble in ether and benzene, and has the crystal form shown in Fig. 1. This product seems to have a pyrimidine ring from its absorption spectra at ultraviolet (Fig. 2) and infrared (Fig. 3) regions, suggestive of an existence of pyrazine ring and a sugar, and from the fact that it produces urea on heating with 1*N* sodium hydroxide. Experiments for establishing its structure are now under way.

Research Laboratory
Takeda Pharmaceutical Industries, Ltd.
Juso, Higashiyodogawa-ku, Osaka

Toru Masuda (増田 亨)

November 25, 1955.

U.D.C. 547.9:582.284

Isolation of some New Substances produced by *Eremothecium ashbyii*

Studies on the mechanism of the biosynthesis of riboflavin were reported by MacLaren,[1] McNutt,[2] Goodwin and Pendlington,[3] and Plaut.[4] To clarify the mechanism, *Ashbya gossypii* or *Eremothecium ashbyii* was cultured in a medium containing purine, pyrimidine, amino acid, or the like and investigated the yield of the resulting riboflavin, or added to the medium adenine or other compounds containing ^{14}C in order to find out which of their parts had taken part in the formation of the riboflavin nucleus. There are two theories about the formation of the riboflavin nucleus. One is the formation of ring-C by the same mechanism as in the formation of the pyrimidine ring of purine, and the other is the formation of ring-A by the rupture of purine ring at C_8 and subsequent addition of an amino acid or the like. However, neither of these theories has been established by the isolation of the intermediate. In the previous paper[5] the writer described about the presence in the mycelium of *E. ashbyii* of a green (G-substance) and a purple (V-substance) fluorescent substances, in addition to riboflavin, adenine, FAD, and ATP. Later the green fluorescent substance was isolated in a crystalline form,[6] followed by the isolation of the purple

1) J. A. MacLaren.: J. Bacteriol., **63**, 233(1952).
2) W. S. McNutt.: J. Biol. Chem., **210**, 511(1954).
3) T. W. Goodwin, S, Pendlington.: Biochem. J.(London), **57**, 631(1954).
4) G. W. E. Plaut.: J. Biol. Chem., **208**, 513(1954).
5) T. Masuda.: This Bulletin, **3**, 434(1956).
6) See the preceding article.

NII-Electronic Library Service