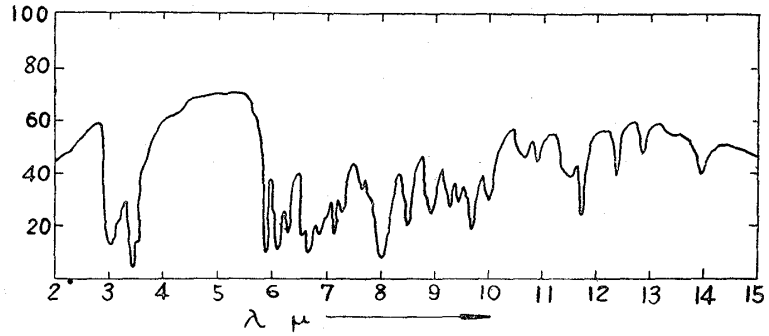

Fig. 3. Infrared Absorption Spectrum of G-Substance (in Nujol)

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.

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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.

fluorescent substance in a crystalline form, and at the same time a new substance was detected.

When the crude G-substance was developed on a column of powdered cellulose with benzyl alcohol, as reported in the preceding paper[6] and then the aqueous extract of the resulting G-substance band was concentrated, yellow crystals separated, leaving the G-substance in the solution. The product gave light yellow needles, m.p. 223~224°(decomp.), $[\alpha]_D^{13}$: −43.2°, having the crystal form shown in Fig. 1, after

Fig. 1

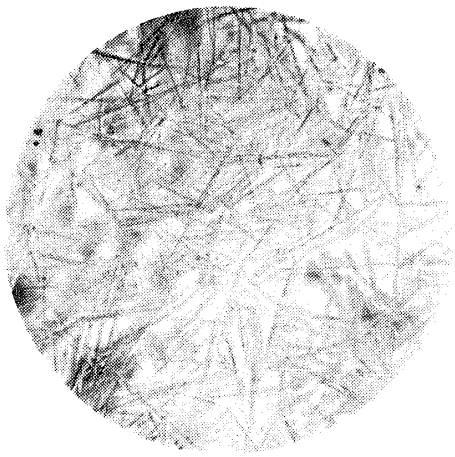

Fig. 2

recrystallization from hot water and had no such fluorescence as the G-substance. Though sparingly soluble in cold water, it dissolves in sodium bicarbonate and sodium hydroxide to form a yellow solution and colorless in acid solution. It is soluble in pyridine and acetic acid but insoluble in ether and benzene. This substance exhibits bluish white fluorescence under ultraviolet rays and gives an orange coloration with dimethylaminobenzaldehyde. It is positive to ninhydrin reaction. *Anal.* Calcd. for $C_{11}H_{14}N_2O_5$: C, 51.96; H,5.52; N, 11.05. Found : C, 51.77; H, 5.89; N, 11.42.

This product shows absorption maxima at 230, 270 and 370 mμ, and its Rf in paper chromatography is at 0.34 when developed with ethanol:butanol:water (15 : 50 : 35), and 0.22 when developed with benzyl alcohol:water. As reported in the previous paper[5] it shows the same Rf as that of riboflavin when developed with ethanol: butanol:water, and the same Rf as that of the G-substance when developed with benzyl alcohol:water, but it can be distinguished from the two compounds by the orange color reaction with dimethylaminobenzaldehyde (both the G-substance and riboflavin are negative to this color reaction). It is evident that this substance is not a decomposition product produced during the purification process, because it is detected on the chromatogram of the aqueous extract of the mycelium obtained from a 40-hour culture of *E. ashbyii*.

The V-substance, of which the writer already reported in the previous paper,[5] was also separated in colorless needles from the aqueous extract of the mycelium of *E. ashbyii*. The extract was first freed from riboflavin and FAD by extraction with benzyl alcohol or by precipitation with hydrosulfite, then subjected to adsorption with Amberlite IR-410 or activated charcoal, followed by extraction with pyridine and then by development on a column of powdered cellulose with 80% ethanol, and finally the substance thus separated was purified by recrystallization (Fig. 2). The yield was only 10 mg. from 10 kg. of the mycelium. The product gave colorless needles, m.p. 239~243°(decomp.), $[\alpha]_D^{20}$: ±0. *Anal.* Calcd. for $C_{12}H_{17}N_4O_7$: C, 43.8; H, 5.17; N, 17.00. Found : C, 43.67; H, 5.17; N, 16.96.

This product shows strong purple fluorescence and, from its ultraviolet and

infrared absorption spectra and from the fact that it gives urea on heating with sodium hydroxide, it seems to have the rings B and C of riboflavin. Studies on its structure are now under way.

The writer has already succeeded in isolating three new substances from the mycelium of *E. ashbyii*, and it was found that the G- and V-substances lack the ring A of riboflavin. If these substances are found to be the intermediates of riboflavin and their structures are clarified, the mechanism of the biosynthesis of riboflavin would become clear.

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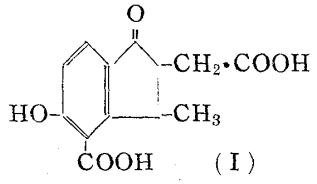
Synthesis of Terracinoic Acid

Terracinoic acid is an acidic compound isolated by Pasternack *et al.*[1] from the alkaline degradation products of Terramycin and its structure was shown to be 4-carboxy-5-hydroxy-3-methylindan-1-one-2-acetic acid (I).[2] The present work was undertaken to provide synthetic confirmation for the structure of this important Terramycin degradation product.



(I)

A reaction of 7-methoxy-3-methylindanone[3] and methyl formate in the presence of sodium methoxide gave 2-hydroxymethylene-7-methoxy-3-methylindanone (II), m.p. 120~122°(decomp.) (Calcd. for $C_{12}H_{12}O_3$: C, 70.59; H, 5.88. Found : C, 70.59; H, 5.84), which was converted by reaction with hydroxylamine hydrochloride in glacial acetic acid to bis(7-methoxy-3-methyl-1-oxo-2-indanylidenemethyl)hydroxylamine (III). Alkaline hydrolysis of (III) yielded β-(2-carboxy-3-methoxyphenyl)buytric acid (IV), m.p. 160.5~161.5°, (Calcd. for $C_{12}H_{14}O_5$: C, 60.50; H, 5.88. Found : C, 59.98; H, 5.84). The dimethyl ester (V), b.p_1 158~160°(Calcd. for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found : C, 62.91; H, 7.00), of (IV), obtained by treatment of (IV) with diazomethane, was partly hydrolyzed with 1 mole of a methanolic solution of *N* sodium hydroxide to β-(2-methoxycarbonyl-3-methoxyphenyl)butyric acid (VI), m.p. 114.5~116.0° (Calcd. for $C_{13}H_{16}O_5$: C, 61.89; H, 6.39. Found : C, 61.78; H, 6.34). Intramolecular acylation of (VI) with polyphosphoric acid afforded 4-methoxycarbonyl-5-methoxy-3-methylindanone (VII), m.p. 106~107°(Calcd. for $C_{13}H_{14}O_4$: C, 66.65; H, 6.02. Found : C, 66.70; H, 5.99), and 4-carboxy-5-methoxy-3-methylindanone (VIII), m.p. 139~140°(Calcd. for $C_{12}H_{12}O_4$: C, 65.44; H, 5.49. Found : C, 65.63; H, 5.30). Bromination of the indanone ester (VII) gave the 2-bromo derivative (IX), m.p. 113.5~114° (Calcd. for $C_{13}H_{13}O_4Br$: C, 49.84; H, 4.15. Found : C, 49.86; H, 4.00), which was converted by reaction with diethyl sodiomalonate to the 2-malonic ester derivative (X), followed by alkaline hydrolysis to

1) R. Pasternack, P. P. Regna, *et al.* : J. Am. Chem. Soc., **73**, 2400(1951); R. Pasternack, A. Bavley, *et al.* : *Ibid.*, **74**, 1926(1952).
2) R. Pasternack, L. H. Conover, *et al.* : *Ibid.*, **74**, 1928(1952).
3) L. H. Conover : *Ibid.*, **75**, 4017(1953).