New Pterin Produced by *Aspergillus*

Sir:

Previously the author and his collaborators have observed fluorescent products in 249 strains of *Aspergillus* belonging to *Asp. flavus*, *oryzae*, *niger*, *tamarii*, *japonicus*, *glaucus*, Awamori-Koji, Shochu-Koji and so on, incubating them in Pfeffer's liquid media and irradiating the broths with ultraviolet lamp.¹⁾

The fluorescent products recognized so far were classified largely into 3 groups, namely (a) yellow fluorescent material, this is riboflavin, (b) markedly bluish purple fluorescent substance, (c) yellowish green fluorescent materials. These are considered to consist of several compounds, to which Aflatoxin²⁾ might possibly belong, if, as in rare case, the *Aspergillus* were a producing strain of it; besides, the other one which was isolated as a crystalline form already by the author.³⁾

In this communication he wishes to deal with this (b) bluish purple fluorescent substance (abbreviated to VS), informing of its production, isolation to a crystalline form and identification as a new pterin.

Asp. oryzae (Ahl.) CONN was incubated in about 1000 l of modified Pfeffer's medium containing 100 g sucrose and 30 g ammonium nitrate per l at 30°C. By stationary culture in duralumin vessel, carbon source was consumed in about 45 days, organic acid products, such as Kojic acid, reached their max. in 45~50 days, then decreased, and mycelium crop yields became max. in about 50 days. VS was formed proportionally to cell growth and did not decrease after attaining to max. amount of VS.

Paper chromatography (*n*-butanol-acetic acid-

water, 4:1:5) of VS showed two fluorescent spots at R_F 0.24 (VS-I) and 0.14 (VS-II).

The broth was filtered after 55 days incubation and the mycelial mat was ground with quartz sand from which the fluorescent materials were extracted with hot water. VS in the filtrate and extract were then adsorbed onto charcoal at pH 7.5. After being eluted from the carbon cake with 0.1 N caustic soda-acetone mixture (4:1), the VS eluates were neutralized immediately and concentrated in vacuo. From the concentrate ether-soluble materials such as yellow pigments and yellowish green fluorescent substances were removed by continuous ether extraction at pH 3.5. VS in aqueous layer were adsorbed again onto charcoal and eluted with alkali acetone similarly as before.

The concentrated eluate was treated with 9 times volume of methanol, VS being dissolved into the 90% methanol layer. The extract was passed through alumina column from which at first the adsorbed VS-I was eluted with 80% methanol, then VS-II with 60% methanol. VS-II fraction was purified once more by similar alumina chromatography.

Dowex-I, X-4, -CO₃ form column chromatography was thereafter applied to VS-II solution which was adsorbed on the top of the column and eluted by hydrochloric acid with batch-wise exchange. Further purification was fulfilled by Filtrol Grade 58 (Adsorbent made by Filtrol Corporation U.S.A.) column chromatography, VS-II having been adsorbed at pH 3.0 and eluted with water containing increasing amount (1%, 2% and so on) of acetone.

VS-II was thus completely separated from minute VS-I contaminant and finally treated with Dowex-I column chromatography again. The eluate was concentrated to yield pale

1) Y. Kaneko, K. Sakaguchi and Y. Kihara, *J. Ferm. Tech.*, **27**, 156 (1949).

2) A. Takagi, and K. Aihara, *J. Food Hygienic Soci. Japan*, **6**, 1 (1965).

3) Y. Kaneko and S. Umemoto, *Proc. 29th Meeting Central Branch Agri. Chem. Soci. Japan*, (1961)

yellow needles of VS-II of about 400 mg.

Recrystallization from hot water afforded fine needles of m.p. 193°C (with decomp.), $[\alpha]_D^{20} -60.2^\circ$ ($c=3.321 \times 10^{-3}$ g/ml, $l=1$ dm). Potentiometric titration of the compound demonstrated pKa 8.0 and m.w. 358.9.

Anal. Found: C, 42.16; H, 5.19; N, 18.23. Calcd. for $C_{13}H_{11}O_7N_5 \cdot H_2O$: C, 41.82; H, 5.13; N, 18.76% m.w. 373.3.

The compound is considerably soluble in water, phenol, moderately soluble in methanol, pyridine, acetic acid, sparingly soluble in ethanol, *n*-butanol, acetone, benzyl alcohol and insoluble in ether, ethylacetate, chloroform, benzene, petroleum ether. Its solution except in phenol exhibits brilliant bluish purple fluorescence especially in alkaline pH water.

The ultraviolet absorption spectra in Fig. 1 shows $\lambda_{\max}$ 259, 281, 360 $m\mu$ in 0.1 *N* sodium hydroxide and 293, 347 $m\mu$ in 0.1 *N* hydrochloric acid. This indicates the fact that VS-II belongs to a typical isoxanthopterin derivative though some bathochromic shift exists.

Basing upon its elemental analysis, m.p. and ultraviolet spectra it is quite obvious that VS-II is a new pterin occurring in nature.

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FIG. 1. Ultraviolet Absorption Spectra of VS-II.

—○— VS-II 1 mg% in 0.1 *N* NaOH
—□— VS-II 1 mg% in 0.1 *N* HCl

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