Note

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Studies on the Components of *Phaeolus schweinitzii* (Fr.) Pat.

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The Basidiomycetes *Phaeolus schweinitzii* (Fr.) Pat. (*Polyporus schweinitzii* Fr.) is harmfull fungus which is parasitic on acerose and decay the timber.

In the preceding paper, Harada, *et al.*[1] reported on the antibacterial activity of the alcohol extract of the fruit body. Bose[2] and Shibata, *et al.*[3] also reported on the antibacterial activity of the culture medium of the mycelium, but did not describe chemical structure of the constituents.

In this laboratory, a kind of pigment was isolated from acetone extract of dried fresh fruit body as yellow needles, $C_{13}H_{10}O_5 \cdot H_2O$, m.p. 246° (decomp.). On heating at 110° in vacuum, it formed a dehydrated compound, $C_{13}H_{10}O_5$. Acetylation of this substance gave diacetate, $C_{17}H_{14}O_7$, m.p. 160°. Alkali fusion of this substance afforded acetone, protocatechualdehyde and caffeic acid. Acid decomposition of this substance with diluted sulfuric acid gave mainly substance A and small amount of substance B. On the paper chromatography, Rf value of the substance A and B showed identity with the other components of this fungus, though these components were not yet isolated in pure state, but their existence was confirmed by the paper chromatography.

During our study was proceeding, Edwards, *et al.*[4] reported on isolation of hispidin from *Polyporus hispidus*, and the structure of hispidin was determined as 4-hydroxy-6-(3,4-dihydroxystyryl)pyrone. Shortly after the Edward's report, Bu'Lock, *et al.*[5] reported on the item and attained the same conclusion.

Physical and chemical properties of hispidin described in these reports were identical with those properties of the pigment isolated from *Phaeolus schweinitzii* (Fr.) Pat. in our laboratory. The isolation method and chemical degradation process, however, were different from those of hispidin and, consequently, degradation products were not same. Then our sample was sent to Dr. Edwards, and was confirmed to be identical with hispidin. Degradation products obtained in our laboratory also afforded supplementary supports to this structure.

The studies on the other components in this fungus including substance A and B are now progressing and will be reported in future.

Experimental[*2]

Isolation of Hispidin——The fresh fruit bodies of *Phaeolus schweinitzii* (Fr.) Pat. collected from *Larix leptolepis* Murray at Mt. Fuji were air dried, and 1.8 kg. of the dried bodies were digested 5

[*1] Oshika, Shizuoka (上野　明, 福島清吾, 斉木保久, (故)原田利一).

[*2] All melting points are uncorrected.

Paper chromatography carried out with the ascending method by using of Toyo filter paper No. 50, and the following solvent systems were used as developer : $Rf_{(1)}$; upper layer of toluene–$CHCl_3$–AcOH–H_2O (3:1:4:1 by volume), $Rf_{(2)}$; lower layer of $CHCl_3$–CH_3NO_2–AcOH–H_2O (2:1:2:1 by volume).

1) T. Harada, M. Mizuno, T. Kato : Yakugaku Zasshi, **72**, 591 (1952).
2) S. R. Bose : T. Sci. Indi. Research (India), **11**, B, 159 (1952) (C. A. **47**, 697 (1953)).
3) S. Shibata, S. Natori : Yakugaku Zasshi, **72**, 594 (1952).
4) R. L. Edwards, *et al.* : J. Chem. Soc., **1961**, 4995, 5003.
5) J. D. Bu'Lock, *et al.* : *Ibid.*, **1962**, 2085.

times with Me_2CO at room temperature. The combined extracts were concentrated to small volume under diminished pressure and then allowed to stand overnight in a refregerator. The yellow crude pigment (25 g.) separated was collected, washed with small portion of Me_2CO and dried. The mother liquor was evaporated. Adding AcOEt to the residue, another portion of crude pigment (15 g.) was separated as yellow solid insoluble to AcOEt. On the paper chromatographical investigation, isolated crude pigment gave main spot at $Rf_{(1)}$ 0.03 and $Rf_{(2)}$ 0.40, and the mother liquor (soluble portion in AcOEt) gave spots of the other components; substance *a*, $Rf_{(1)}$ 0.31 or $Rf_{(2)}$ 0.83, *b*, $Rf_{(1)}$ 0.19 or $Rf_{(2)}$ 0.74, *c*, $Rf_{(2)}$ 0.54, *d*, $Rf_{(2)}$ 0.30 and *e*, $Rf_{(2)}$ 0.09. A saturated solution of crude pigment in Me_2CO was passed through a column of $CaHPO_4$ and the chromatogram was developed with Me_2CO. The eluate corresponds to second yellow zone was collected, evaporated to dryness, and the residue was recrystallized from 60% EtOH to form yellow needles, m.p. 246~250° (decomp.), yield, 22 g. (1.2%). UV λ_{max}^{EtOH} mμ (log ε): 206 (4.48), 223 (4.50), 253 (4.14), 366 (4.24). *Anal.* Calcd. for $C_{13}H_{10}O_5 \cdot H_2O$: C, 59.09; H, 4.58; H_2O, 6.28. Found: C, 59.08; H, 4.52; H_2O, 6.71. The product was confirmed to be identical with authentic hispidin*3 by an admixture and comparison of the IR spectra. Hispidin gave the dehydrated compound by drying on P_2O_5 in vacuum at 110°. IR ν_{max}^{KBr} cm^{-1} hispidin (dehydrated form): 3500 (3300), 1690 (—), 1660 (1656), 1117 (1125), 812 (s) (805~825 (m)). *Anal.* Calcd. for $C_{13}H_{10}O_5$: C, 63.41; H, 4.09. Found: C, 63.34; H, 4.03. It was reconverted to hydrated form by recrystallization from 60% EtOH.

O,O-Diacetylhispidin——A mixture of AcOCl (2 ml.), pyridine (5 ml.), and AcOH (5 ml.), mixed at 0°, was added to a solution of pigment (0.2 g.) in pyridine (2 ml.) at 0°. After standing for 5 min. at 0°, the mixture was poured into cold H_2O (50 ml.), and AcONa (1 g.) was then added. Separated crystals were recrystallized four times from EtOH to yield pale yellow plates (0.15 g), m.p. 224°. *Anal.* Calcd. for $C_{17}H_{14}O_7$: C, 61.82; H, 4.27. Found: C, 61.59; H, 4.21. The product was confirmed to be identical with authentic O,O-diacetylhispidin*3 by a mixed fusion and comparison of the IR spectra.

O,O,O-Trimethylhispidin——To a suspension of pigment (0.2 g.) in MeOH (4 ml.), an ethereal solution of CH_2N_2 (prepared from 2 g. of N-methyl-N-nitrosourea) was added. After standing the mixture overnight at room temperature, separated crystals were collected by decantation of solvent, washed with MeOH, and recrystallized three times from MeOH to give pale yellow needles (0.18 g.), m.p. 160°. *Anal.* Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.33; H, 5.48. The product was confirmed to be identical with authentic O,O,O-trimethylhispidin*3 by an admixture and IR spectrum determination.

Alkali Fusion of Hispidin——Hispidin (2 g.) was added to the fused mixture (150~160°) of KOH (4 g.) and H_2O (2 ml.), and the temperature was kept at 150~160° for 20 min. in H_2 current. After cooling, the mixture was added with 50% KOH solution (1 ml.), and heated for 10 min. under the same condition. During the reaction, volatile product was passed through Brady's reagent, from which crystals were separated. Recrystallization of this crystals from MeOH afforded yellow prisms, m.p. 125°. *Anal.* Calcd. for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.52. Found: C, 45.45; H, 4.23; N, 23.32. On admixture with acetone 2,4-dinitrophenylhydrazone, m.p. 125°, it showed no depression of melting point. After cooling the reaction mixture was dissolved in H_2O, saturated with CO_2 and then extracted with Et_2O (phenolic portion). The H_2O layer was acidified and shaken with Et_2O (acid portion).

Phenolic portion: The residue obtained on evaporation of Et_2O, colored green with $FeCl_3$ and recrystallization from benzene-AcOEt mixture afforded colorless needles, m.p. 155°. *Anal.* Calcd. for $C_7H_6O_3$: C, 60.87; H, 4.38. Found: C, 60.78; H, 4.41. It showed identity with protocatechualdehyde, m.p. 155°, by a mixed fusion and comparison of the IR spectra.

Acid portion: After evaporating of Et_2O, the residue was recrystallized from H_2O to form colorless needles, m.p. 213°. *Anal.* Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.48. Found: C, 59.71; H, 4.42. It was identified with caffeic acid by a mixed fusion and comparison of the IR spectra.

Acid Decomposion of Hispidin——A suspension of powdered hispidin (1 g.) in 10% H_2SO_4 (300 ml.) was heated on a boiling water bath for 4 hr. in N_2 atmosphere. After cooling the reaction mixture was extracted with AcOEt, and the AcOEt-extract was washed with H_2O, and dried over Na_2SO_4 and concentrated to small volume and then passed through a column of $CaHPO_4$. The residue obtained on evaporation of first eluate was dissolved in small amount of Me_2CO. To this solution, powder of $CaHPO_4$ was added, and Me_2CO was evaporated to dryness. The resulting $CaHPO_4$ powder was piled on a column of $CaHPO_4$ covered with benzene and chromategraphed using benzene-Me_2CO mixture with gradually raising contents of Me_2CO from 1% to 10% for the developer. By paper chromatography, the eluted portion was tested in each fraction, and eluate A, which gave a spot of $Rf_{(1)}$ 0.31, and eluate B, which gave a spot $Rf_{(1)}$ 0.19, were collected.

Substance A: The residue obtained on evaporation of solvent from eluate A was recrystallized from CH_3NO_2 to yield 350 mg. of substance A as yellow needles, m.p. 154°. It showed a green color with ethanolic $FeCl_3$. *Anal.* Calcd. for $C_{12}H_{12}O_4$: C, 65.44; H, 5.49. Found: C, 65.30; H, 5.56. On the paper chromatographical investigation, the spot of this substance was compared with the spot of

*3 All of these authentic samples were furnished through the courtest of Dr. Edwards.

substance *a* described in the case of isolation. Both spot revealed at $Rf_{(2)}$ 0.83 and $Rf_{(1)}$ 0.31, appearing yellow brown on spraying 1% Na_2CO_3.

O,O-Diacetate of substance A : A solution of substance A (0.1 g.) in pyridine (1 ml.) and a mixture of AcOCl (1 ml.), pyridine (1 ml.), and AcOH (3 ml.) was treated with the same method used in the case of O,O-diacetylhispidin. Recrystallization of the product from MeOH gave pale yellow plates, m.p. 121°. It showed a deep red color with ethanolic $FeCl_3$. *Anal.* Calcd. for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found : C, 62.92; H, 5.30.

Substance B : Substance B (80 mg.) was obtained from eluate B on recrystallization from CH_3NO_2 as brown prisms, m.p. 177~178°. On the paper chromatographical investigation, the spot of this substance was compared with the spot of substance *b* described in the case of isolation. Both spot revealed at $Rf_{(2)}$ 0.74 and $Rf_{(1)}$ 0.19, appearing brown on spraying 1% Na_2CO_3.

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Summary

Chemical components of *Phaeolus schweinitzii* (Fr.) Pat. were studied. Yellow pigment isolated from the fruit body was confirmed to be identical with hispidin.

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