Chem. Pharm. Bull.
26(4)1320—1322(1978)

UDC 547.97.02.05 : 547.861.1.02 : 581.192

Isolation and Structure of Red Pigment from *Aspergillus ochraceus* Wilh[1]

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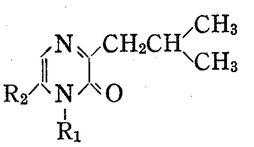
(Received September 9, 1977)

A red pigment which was isolated from cultures of *A. ochraceus* Wilh. IFM 4443 gave neoaspergillic acid (II) and ferric hydroxide by hydrolysis. The mass spectrum of this pigment suggested that three molecules of neoaspergillic acid and one atom of iron made the pigment. β-Hydroxyneoaspergillic acid (V) was also obtained from the same fungus. Its structure was deduced by its spectral evidence and by reduction to deoxy-β-hydroxyneoaspergillic acid (IV).

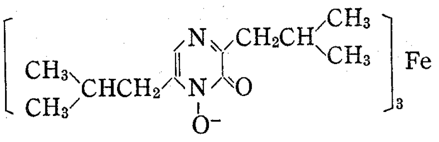
Keywords——structure elucidation; red pigment; neoaspergillic acid; iron complex; β-hydroxyneoaspergillic acid; *Aspergillus ochraceus*; fungi

The isolation of a new pyrazine compound, deoxy-β-hydroxyneoaspergillic acid (IV) from *Aspergillus ochraceus* Wilh. IFM 4443 together with flavacol (I) and neoaspergillic acid (II) was previously reported.[3] The fungus was also found to produce ochratoxins,[4] emodin,[5] secalonic acid,[5] a new isocoumarinic acid[6] and a red pigment.[3]

In this brief report, the structure elucidation of the red pigment (VI) and a pyrazine compound newly isolated from the fungus is described.



I: R_1=H, R_2=$CH_2CH(CH_3)_2$
II: R_1=OH, R_2=$CH_2CH(CH_3)_2$
III: R_1=OH, R_2=$CH(OH)CH(CH_3)_2$
IV: R_1=H, R_2=$CH_2C(OH)(CH_3)_2$
V: R_1=OH, R_2=$CH_2C(OH)(CH_3)_2$



VI

Red pigment (VI), mp 135—136°, has been isolated from mycelia by silica gel column chromatography (Chart 1). By treatment with 2N NaOH–MeOH, VI gave dark brown precipitates of ferric hydroxide. From the filtrate, colorless needles, mp 123—125°, were obtained, which were identified with authentic neoaspergillic acid (II). Accordingly, VI was proposed as a complex salt of neoaspergillic acid with iron. In the mass spectrum (MS) of this pigment, three strong peaks at *m/e* 725.5 (M^+), 502, and 223 were observed, but only one peak of the molecular ion at *m/e* 725.5 was observed in the field desorption MS of this compound. This result thus indicated that three molecules of neoaspergillic acid (II) chelated with one atom of iron to make a red complex salt. It is likely proposed to this pigment to call ferrineoaspergillin (VI).

From the culture filtrates, colorless amorphous compound (V), mp 143—144°, $C_{12}H_{20}N_2O_3$, was obtained by silica gel column chromatography (Chart 2). This compound gave red color for ferric chloride test and green copper salt (mp 241—244°) with copper sulphate. The

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[1] A part of this study was reported at 17th Annual Meeting of Kanto Branch, Pharmaceutical Society of Japan (1973, Tokyo).
[2] Location: *Inohana, Chiba-shi, Chiba.*
[3] M. Yamazaki, Y. Maebayashi, and K. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2274 (1972).
[4] M. Yamazaki, Y. Maebayashi, and K. Miyaki, *Appl. Microbiol.*, **20**, 452 (1970).
[5] M. Yamazaki, Y. Maebayashi, and K. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 199 (1971).
[6] M. Yamazaki, Y. Maebayashi, and K. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2276 (1972).

Ultraviolet (UV) spectrum of V, $\lambda_{\max}^{\text{EtOH}}$ nm (ε) 237 (16238), 333 (12511), was similar to those of neoaspergillic acid (II) and its related compounds. The nuclear magnetic resonance and mass spectrum of V suggested that this compound had a similar side chain to those of compound IV. Catalytic reduction of V over Raney-Ni afforded a product, mp 112—117°, which was confirmed to be identical with deoxy-β-hydroxyneoaspergillic acid (IV) previously isolated in our laboratory[3] and synthesized by Ohta *et al.*[7] From these results, the structure of this compound could be expected as V.

Colorless platelets, mp 157—162°, $C_{12}H_{20}N_2O_3$, isolated from another fraction were deduced to be a known pyrazine compound, neohydroxyaspergillic acid (III) by comparison of the nuclear magnetic resonance (NMR) spectrum with those reported.[8]

Thus, from a strain of *A. ochraceus* Wilh. IFM 4443, six neoaspergillic acid related metabolites, flavacol (I), neoaspergillic acid (II), neohydroxyaspergillic acid (III), deoxy-β-hydroxyneoaspergillic acid (IV), β-hydroxyneoaspergillic acid (V) and ferrineoaspergillin (VI), have been isolated. All of these compounds are the metabolites derived from two molecules of leucine. The isolation of differently hydroxylated pyrazines from the same fungus is first resulted in this investigation. One is hydroxylated on α-position of one of the isobutyl side chain and the other is on β-position.

The fact suggests the presence of following hydroxylation process on side chain in the biosynthesis of these metabolites: hydroxylation on α-position→dehydration→rehydration (hydroxylation on β-position *via* epoxidation and reduction?). It is of interest that no other type pyrazines which would be derived from isoleucine-isoleucine, isoleucine-leucine and valine-leucine have been isolated from this fungus.

Experimental

Microorganism——*Aspergillus ochraceus* Wilh. IFM 4443 was isolated from moldy rice and maintained on malt extract and Czapeck agar containing 20% sucrose in this laboratory. The fungus was cultivated as previously reported.[4]

Isolation of V and VI——Isolation of the pyrazine metabolites was carried out according to the procedure illustrated in Chart 1 and 2.

Red Pigment (VI)——Red leaflets, mp 135—136° from acetone–H_2O. Soluble in most organic solvent but insoluble in water. UV $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 227 (49541), 315 (18125), 410 (4350). IR KBr cm^{-1}: 3440, 2955, 2860, 1523, 1488. MS *m/e* (%): 725.5 (100), 502 (100), 223 (53). Field desorption (FD) MS: 725.5 (M^+). By treatment with 2 N NaOH (20 ml)–MeOH (10 ml), VI (206 mg) gave dark brown precipitates which was dissolved in conc.-HCl and determined as ferric hydroxide by coloring (Berlin blue) with $K_4Fe(CN)_6$. From the filtrates, colorless needles (135 mg) mp 126—127.5° (from ethanol) were obtained and identified with authentic neoaspergillic acid (II) by comparison of the spectra data and mixed fusion.

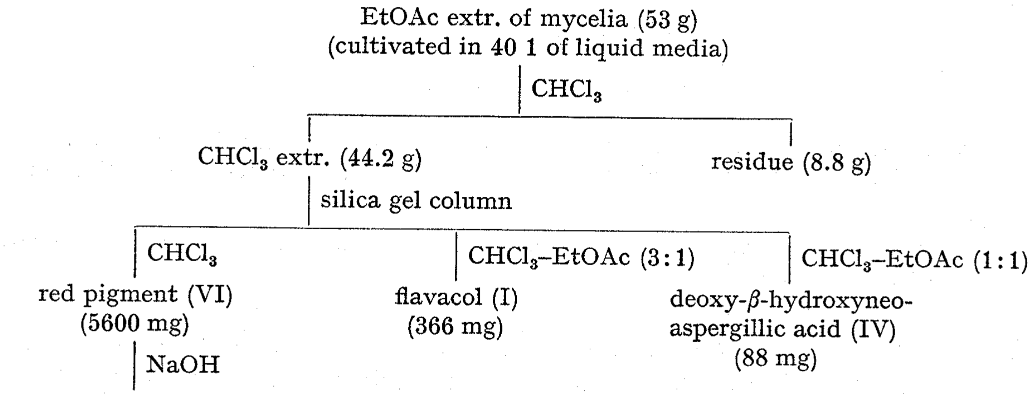

Chart 1

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[7] This work was reported at 93rd Annual Meeting of the Pharmaceutical Society of Japan, April, 1973.

[8] R.G. Micetich and J.C. MacDonald, *J. Chem. Soc.*, **1964**, 1507.

Neoaspergillic Acid (II)——Colorless needles, mp 126—127.5° from ethanol. Reddish brown with $FeCl_3$. UV $\lambda_{\max}^{\text{EtOH}}$ nm (ε) 234 (10557), 330 (8143). IR KBr cm^{-1}: 3430, 2960, 2440, 2040, 1638, 1584, 1567, 1240, 1157. NMR in $CDCl_3$ δ ppm: 0.99 (6H, doublet, $J=7.0$), 0.94 (6H, doublet, $J=7.0$), 1.96—2.44 (2H, multiplet), 2.67 (4H, triplet, $J=7.0$), 9.71 (1H, broad singlet). MS m/e (%): 224(30), 207(63), 193(28), 166(40), 153(43), 123(61), 43 (23).

β-Hydroxyneoaspergillic Acid (V)——Colorless amorphous, mp 143—144° (from ethylacetate). $C_{12}H_{20}N_2O_3$ (m/e Found: 240.146, Calcd: 240.1473). UV $\lambda_{\max}^{\text{EtOH}}$ nm (ε): 237 (16238), 333 (12511). IR KBr cm^{-1}: 3350, 2955, 2925, 2870, 2060, 1640, 1575, 1235, 990, 720. NMR in $CDCl_3$ δ ppm: 0.95 (6H, doublet, $J=6$), 1.34 (6H, singlet), 2.18 (1H, multiplet), 2.69 (2H, doublet, $J=8$), 2.99 (2H, singlet), 7.39 (1H, singlet). MS m/e (%): 240(M^+, 20), 182(38), 166(32), 123(100), 59(100), 43(38), 41(31). Catalytic reduction of V over Raney-Ni in MeOH at room temperature gave crystals (mp 112—117°) by following purification through silica gel column chromatography. The product was negative for $FeCl_3$ test and its UV spectrum was identical with those of IV. Identified with authentic deoxy-β-hydroxyneoaspergillic acid by mixed fusion.

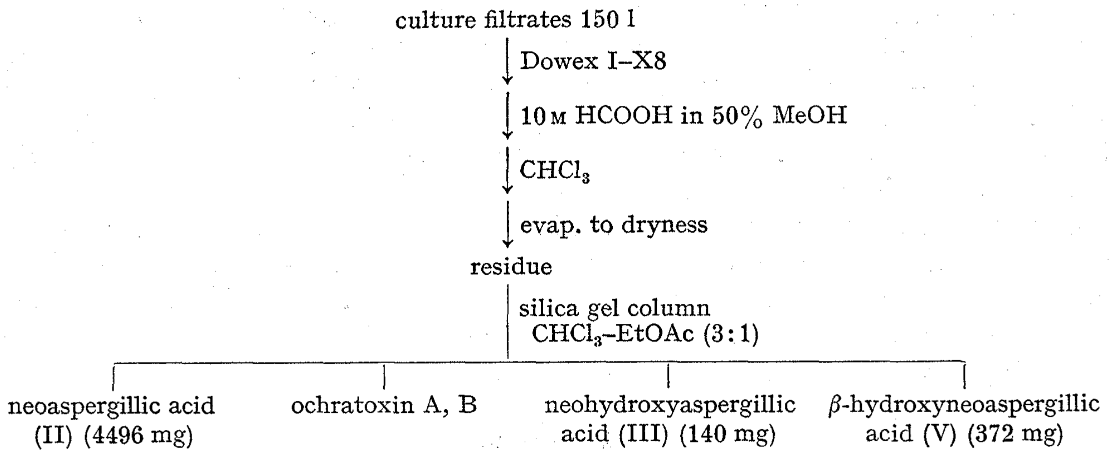

Chart 2

Chem. Pharm. Bull. 26(4)1322—1325(1978)

UDC 547.861.2.04 : 547.233.2'546.18.04

Reaction of Some Halogenated Aromatic N-Heterocycles with Hexamethylphosphoric Triamide

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(Received September 16, 1977)

Some halogenated pyrazines, pyridines, and quinolines were heated with hexamethylphosphoric triamide at 200° to yield the corresponding dimethylamino derivatives in a high yield.

Keywords——HMPA; halogeno N-heterocycles; pyrazine; pyridine; quinoline; dimethylamino N-heterocycles

Although hexamethylphosphoric triamide (HMPA) is one of the most widely used polar solvents, it is also applicable to dimethylamination reaction. As reported by Pedersen and his co-workers, potential substituents[2] of some benzene derivatives and 2-hydroxyl group[3]

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[1] Location: *1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.*

[2] E.B. Pedersen, J. Perregard, and S.-O. Lawesson, *Tetrahedron*, 29, 4211 (1973).

[3] E.B. Pedersen and S.-O. Lawesson, *Tetrahedron*, 30, 875 (1974).