**EtOAc extr. of mycelium**
(cultivated in 40 liter of liquid media) (53 g)

- CHCl₃
  - CHCl₃ extr. (44.2 g)
  - residue (8.8 g)
  - silica gel column
    - CHCl₃
    - CHCl₃-EtOAc (3:1)
    - CHCl₃-EtOAc (1:1)
  - AO-1
    - NaOH
    - AO-1'
  - AO-2
    - (366 mg)
  - AO-3
    - (88 mg)

**Chart 1**

**Deoxyne-β-hydroxyaspergilliac Acid (AO-3) (I)**—Colorless needles, mp 122.5—123° from ethyl acetate. Pale purple fluorescence under UV light. C₁₈H₁₉O₂N₂. Found: C, 64.49; H, 8.85; N, 12.55. Calcd: C, 64.25; H, 8.99; N, 12.49. UV λ max nm (ε) 230 (5016), 326.5 (5528). IR KBr cm⁻¹: 3290, 2945, 1907, 1634, 1520, 1464, 1304. NMR in d-pyridine 0 ppm 0.99 (6H, doublet, J=6.5), 1.37 (6H, singlet), 2.47 (1H, multiplet), 2.75 (2H, singlet), 2.89 (2H, doublet, J=6.5), 7.40 (1H, singlet). Mass Spectrum m/e: 224 (M⁺), 182, 166, 124, 123, 59 (base peak).

**Acknowledgement** Thanks are due to Dr. A. Ohta of Tokyo Woman’s Colledge of Pharmacy for a gift of the authentic samples of flavacol and neosaspergillacic acid.

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**Isolation of a New Metabolite, 6-Methoxy-8-hydroxyisocoumarin-3-carboxylic Acid from Aspergillus ochraceus Wilh.**

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The isolation of pyrazine metabolites from Aspergillus ochraceus Wilh. IFM 4443 has been reported in our preceding paper.² Besides above pyrazines, a colorless crystalline compound has now been isolated by silica gel column chromatography of chloroform extract from culture filtrates of the same fungus. By culturing the fungus in 40 liter of liquid media,³ 32 mg of the compound was yielded.

The compound (Ia) was obtained as colorless needles, mp >300° by recrystalization from methanol. Blue fluorescence was shown under ultraviolet (UV) light but negative with FeCl₃. From the resemblance of the character on thin-layer chromatography including its fluorescent property and the fact that the various isocoumarin metabolites have been isolated from this fungus, Ia seems to be an isocoumarin derivative. The UV spectrum of Ia was

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1) Location: Izumi-cho, Narashino-shi, Chiba.
slightly different from that of the typical hydroxyisocoumarins, e.g. of canescin\textsuperscript{4} or reticulol\textsuperscript{5}, suggesting that Ia had a minor structural difference in the molecule. The presence of the carboxyl group in it was shown in infrared spectrum and by the positive Gries reaction. Methylation of Ia with diazomethane afforded a methyl ester (Ib), and in the nuclear magnetic resonance (NMR) spectrum of which compound, signals showing the presence of additional two O-methyls were observed other than the original one in Ia. Instead, a signal at 10.89 ppm of the chelated hydroxyl proton in Ia was disappeared. Simultaneously, absorption at 1680 cm\textsuperscript{-1} (chelated carboxyl of the lactone) in infrared absorption spectrum of Ia was shifted to 1708 cm\textsuperscript{-1} by methylation, indicating that the structure of -OH...O=C was present in Ia. On the position of the original methoxy in Ia, the observation of two aromatic protons meta-coupled each other in NMR-spectrometry supported the expectation that the methoxy would be attached at 6. Further, in NMR spectrum of Ib, a singlet of one proton was observed at 7.58 ppm, suggested that the position bearing this proton was 4 but not 3. As a fact supporting the conclusion as mentioned above, in NMR-study on oosponol (II), Yamamoto, et al.\textsuperscript{6} reported the chemical shift of the proton attached at 3 was as low as 8.23 ppm. In mass spectrometry of Ia, m/z 236 (M\textsuperscript{+}, 88%), 191 ([M-CO\textsubscript{2}H\textsubscript{4}]\textsuperscript{+}, 100%), and 135 (47%) were mainly observed, and of Ib, 264 (M\textsuperscript{+}, 78%), 205 ([M-CO\textsubscript{2}H\textsubscript{4}]\textsuperscript{+}, 100%), and 149 (50%) were observed. Also an accurate mass determination of M\textsuperscript{+} in mass spectrometry of Ib established the molecular formula, C\textsubscript{13}H\textsubscript{12}O\textsubscript{6} for it, and accordingly, C\textsubscript{11}H\textsubscript{8}O\textsubscript{6} was given for Ia.

Conclusively, the structure Ia could be expected for the metabolite newly isolated from the fungus and Ib for its methyl derivative.

Experimental

**Microorganism** — *Aspergillus ochraceus* W\textsubscript{H}. IFM 4443.\textsuperscript{5}

**Isolation of Ia** — Ia was isolated according to the procedure illustrated in Chart 1.

Ia — Colorless needles, mp>300° from methanol. Insoluble in most organic solvents. Slightly soluble in methanol and ethanol. Blue fluorescence under UV-light. UV \textsubscript{max} nm (e) 252(15301), 302(1818), 308

CH\textsubscript{3}Cl extr. of culture liquid (40 liter) (24 g)

\[
\text{CHCl}_3
\]

sol.

residue

evap.

residue (15 g)

petr. ether

sol.

residue (12.4 g)

silica gel column

CHCl\textsubscript{3}–EtOAc (1:1)

Ia (32 mg)

Chart 1


(2144), 339(2465). IR$\mathrm{KBr}$, cm$^{-1}$ 3230, 3060, 2820—2000, 1715, 1680, 1605, 1440, 1355, 1190, 1165. NMR in d-DMSO $\delta$ ppm 3.89 (3H, singlet), 6.69 (1H, doublet, $J = 3.0$), 6.94 (1H, doublet, $J = 3.0$), 7.58 (1H, singlet), 10.89 (1H, singlet). Mass Spectrum m/e: 236 (M$^+$, 88%), 191 (100%), 135 (47%).

**Methylation of Ia**——Suspended 15 mg of Ia in 50 ml of methanol and added ether solution of diazomethane upon it. After 1 day, solvent was evaporated and 16 mg of Ib was obtained. Recrystallized from methanol.

**Ib**——Colorless needles, mp 203—204$^\circ$ from methanol. Insoluble in most organic solvents. Slightly soluble in methanol and ethanol. IR$\mathrm{KBr}$, cm$^{-1}$ 3090, 2940, 1733, 1708, 1595, 1464, 1370, 1283, 1199. NMR in d-DMSO $\delta$ ppm 3.88(3H, singlet), 3.92 (6H, singlet), 6.80(1H, doublet, $J = 2.5$), 7.01(1H, singlet). Mass Spectrum m/e: 264.0625 (Calcd.: 264.2370 (M$^+$, 78%), 205(100%), 149(50%).

**Acknowledgement** We are indebted to Prof. Y.Yamamoto of Kanazawa Univ. for supplying the sample of oospoic acid for NMR-comparison.

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**On the Constituents of Seeds of Horsfieldia iryagchedhi Warb. I**

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The seeds of *Horsfieldia iryagchedhi* Warb. (=*Myristica horsfieldia*, *M. iryagchedhi*) (Myristicaceae), an indigenous plant to Ceylon, are about 1.5 inches of oblong shape and are little smaller in size than the seeds of *Myristica fragrans* Houtt. (nutmegs). Although the chemical constituents of nutmegs have been investigated extensively due to its pharmaceutical necessity (*e.g.* as an ingredient in Aromatic Rhubarb Tincture or a condiment), no work has been provided on the seeds of *H. iryagchedhi*. The present paper is concerned with the isolation of $d$-asarin (I) and dodecanoylphloroglucinol (IIa) from the seeds.

The fractionation was undertaken as shown in Chart 1. The unsaponifiable portion obtained from the neutral fraction afforded a substance, mp 122.5—123, whose physical data (ultraviolet (UV), infrared (IR), proton magnetic resonance (PMR), and mass spectra, and $[\alpha]_b$) are in good accord with those of $d$-asarin (I), and it was identified with the authentic sample by the direct comparison. Minute examination of the cold methanol extract of the seeds by thin-layer chromatography (TLC) disclosed that the seeds do not contain $d$-sesamin, which has been known to isomerize to $d$-asarinin acid treatment at reflux. The saponifiable portion was dissolved to consist of myristic and lauric acids by mass spectrometry and gas-liquid chromatography (GLC, as methyl esters). The alkali soluble fraction