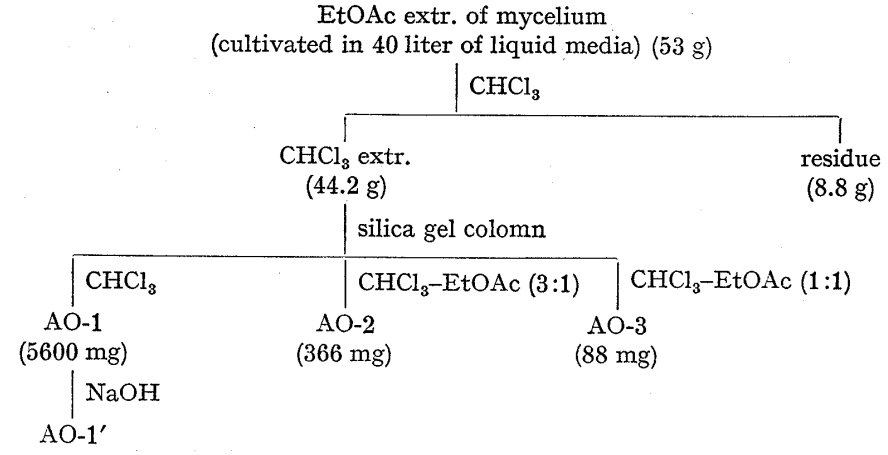

Chart 1

Deoxyneo-β-hydroxyaspergillic Acid (AO-3) (I)——Colorless needles, mp 122.5—123° from ethyl acetate. Pale purple fluorescence under UV light. $C_{12}H_{20}O_2N_2$. Found: C, 64.49; H, 9.35; N, 12.55. Calcd: C, 64.25; H, 8.99; N, 12.49. UV λ_{max}^{EtOH} nm (ε) 230 (5016), 326.5 (5528). IR $_{KBr}$ cm^{-1}: 3290, 2945, 1907, 1634, 1520, 1464, 1364. NMR in *d*-pyridine δ 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 neoaspergillic 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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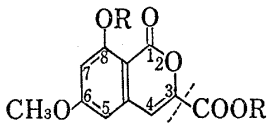
The isolation of pyrazine metabolites from *Aspergillus ochraceus* Wilh. IFM 4443 has been reported in our preceding paper.[2] Besides above pyrazines, a colorless crystalline compound has now been isolated by silica gel column chromatography of chloroform extract from culture filtrates of the same fungus. 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 $FeCl_3$. 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

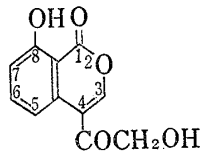
1) Location: *Izumi-cho, Narashino-shi, Chiba.*
2) M. Yamazaki, Y. Maebayashi and K. Miyaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 2274 (1972).
3) M. Yamazaki, Y. Maebayashi and K. Miyaki, *Appl. Microbiol.*, **20**, 452 (1970).

slightly different from that of the typical hydroxyisocoumarins, *e.g.* of canescin[4] or reticulol,[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^{-1} (chelated carbonyl of the lactone) in infrared absorption spectrum of Ia was shifted to 1708 cm^{-1} by methylation, indicating that the structure of -OH···O=C was present in Ia. On the position of the original methoxyl in Ia, the observation of two aromatic protons *meta*-coupled each other in NMR-spectrometry supported the expectation that the methoxyl would be attached at 6. Further, in NMR spectrum of Ia, 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.*[6] reported the chemical shift of the proton attached at 3 was as low as 8.23 ppm. In mass spectrometry of Ia, *m/e* 236 (M^+, 88%), 191 ($(M-CO_2H)^+$, 100%), and 135 (47%) were mainly observed, and of Ib, 264 (M^+, 78%), 205 ($(M-CO_2CH_3)^+$, 100%), and 149 (50%) were observed. Also an accurate mass determination of M^+ in mass spectrometry of Ib established the molecular formula, $C_{13}H_{12}O_6$ for it, and accordingly, $C_{11}H_8O_6$ 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.



Ia : R=H
Ib : R=CH_3



II

Experimental

Microorganism——*Aspergillus ochraceus* WILH. IFM 4443.[2]

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 λ_{max}^{EtOH} nm (ε) 252(15301), 302(1818), 308

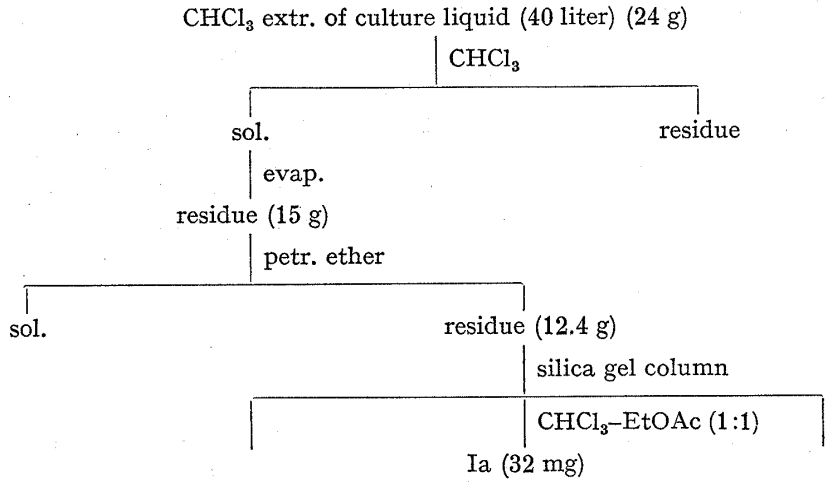


Chart 1

4) A.J. Birch, L. Loh, A. Peter, J.H. Birkinshaw, P. Chplan, A.H. Manchanda, and M. Riano-Martin, *Aust. J. Chem.*, **22**, 1933 (1969).
5) L.A. Mitscher, W.W. Andres, and W.McCrae, *Experientia*, **20**, 258 (1964).
6) I. Yamamoto, K. Nitta, and Y. Yamamoto, *Agr. Biol. Chem.*, **27**, 817 (1963).

(2144), 339(2465). IR_{KBr} cm^{-1} 3230, 3060, 2820—2000, 1715, 1680, 1605, 1440, 1355, 1190, 1165. NMR in *d*-DMSO δ 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. Recrystalized from methanol.

Ib——Colorless needles, mp 203—204° from methanol. Insoluble in most organic solvents. Slightly soluble in methanol and ethanol. UV λ_{max}^{EtOH} nm (ε) 254.5(35494), 309.5(3509), 327(2948), 339(2679). IR_{KBr} cm^{-1} 3090, 2940, 1733, 1708, 1595, 1464, 1370, 1283, 1199. NMR in *d*-DMSO δ 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 suppling the sample of oospoic acid for NMR-comparison.

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

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The seeds of *Horsfieldia iryaghedhi* Warb. (=*Myristica horsfieldia*, *M. iryaghedhi*) (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* Houttuyn (nutmegs). Although the chemical constituents of nutmegs have been investigated extensively due to its pharmaceutic necessity (*e.g.* as an ingredient in Aromatic Rhubarb Tincture or a condiment),[2] no work has been provided on the seeds of *H. iryaghedhi*. The present paper is concerned with the isolation of *d*-asarinin (I) and dodecanoylphloroglucinol (IIa) from the seeds.[3]

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 $[\alpha]_D$) are in good accord with those of *d*-asarinin (I),[4] and it was identified with the authentic sample[5] 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[5,6] which has been known to isomerize to *d*-asarinin on acid treatment at reflux.[4b] The saponified portion was disclosed to consist of myristic and lauric acids by mass spectrometry and gas-liquid chromatography (GLC, as methyl esters). The alkali soluble fraction

1) Location: *a*) *Toneyama, Toyonaka, Osaka*; *b*) *Peradeniya, Ceylon.*
2) E.P. Claus and V.E. Tyler, Jr., "Pharmacognosy," 5th ed., Lea & Febiger, Philadelphia, 1965, p. 223.
3) Presented at the 21st Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka November 1971, Abstract Papers, p. 22.
4) *a*) E.D. Becker and M. Beroza, *Tetrahedron Letters*, **1962**, 157; *b*) K. Takahashi and T. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 641 (1966); *c*) A. Pelter, *J. Chem. Soc.* (*C*), **1967**, 1376; *d*) A.M. Duffield, *J. Heterocyclic Chem.*, **4**, 16 (1967); *e*) L.V. Tutupalli and M.G. Chaubal, *Phytochemistry*, **10**, 3331 (1971).
5) Kindly provided by Prof. K. Takahashi of Kanazawa University.
6) W.A. Jones, M. Beroza, and E.D. Becker, *J. Org. Chem.*, **27**, 3232 (1963).