Although the separation could be carried out as shown in the separate paper,[8] the yield of each compound was determined by comparing intensities of respective signals in the NMR spectrum of the mixture as 12.9 and 16.3%.

Photo-rearrangement of Indole-1-acetamide (VII)——A solution of 148 mg (0.85 mmole) of indole-1-acetamide (VII) in 85 ml of 20% aqueous ethanol was irradiated with 10 W low pressure mercury lamp for 12 hr. Three batches totaling a volume of 255 ml were combined and concentrated *in vacuo* to a volume of *ca.* 30 ml, to which 2 g of sodium hydroxide in 10 ml of ethanol was added and heated at 100° for 1.5 hr. After evaporation of the ethanol, the solution was acidified by the addition of 10% hydrochloric acid under ice-cooling to precipitate a pale red solid, which was extracted with ether. The ether extract was washed with water, dried over sodium sulfate and concentrated to leave a pale red crystalline powder, which was esterified by the treatment with diazomethane in ether. Evaporation of the ether left 303 mg of a pale brown oil. The yield of each compound was determined in the same manner to the preceding experiment.

Photo-rearrangement of Methyl Indoline-1-acetate (X)——A solution of 164 mg (0.86 mmole) of methyl indoline-1-acetate (X) in 85 ml of ethanol was irradiated with 10 W low pressure mercury lamp under nitrogen for 17 hr. Two batches were combined and the solvent was evaporated *in vacuo* to leave a deep brown tarry oil, which was dissolved in methylene chloride and passed over a short column of 4 g of silica gel. Evaporation of the methylene chloride left 262 mg of a deep brown oil, which was chromatographed on a column of 20 g of silica gel. Elution with methylene chloride gave 20 mg (10%) of indole, 16.5 mg (5%) of the starting material and 10.6 mg (3.26%) of methyl indole-5-acetate (VIIId). Their structures were confirmed by comparing with the authentic sample in IR, NMR and TLC.

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Isolation of a New Type of Pyrazine Metabolite from *Aspergillus ochraceus* Wilh.[1]

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We now would like to report briefly that a new type of pyrazine metabolite (I) which is hydroxylated on β-position of an isobutyl side chain has first been isolated as a fungal product from a strain of *A. ochraceus*[3] together with flavacol[4] and neoaspergillic acid.[5]

Colorless needles of I, mp 122.5—123° were afforded by crystallization from ethyl acetate of an eluate with $CHCl_3$ in silica gel chromatography and its molecular formula, $C_{12}H_{20}O_2N_2$ was given by mass spectrometry (M^+ *m/e* 224) and elementary analysis (found: C, 64.49%; H, 9.35%; N, 12.50%). Pale purple fluorescence was observed under ultraviolet light but negative with $FeCl_3$ solution. Ultraviolet absorption spectrum of I in ethanol showed a close resemblance to that of flavacol in which the absorption maxima at 230 (ε 5016) and 326.5 (ε 5528) nm were observed. The presence of hydroxyl and amide groups was suggested by infrared absorption spectrum: IR_{KBr} cm^{-1} 3290, 2945, 1907, 1634, 1520, 1464, 1364, 1174. It was thus indicated from above spectral data that 2-hydroxypyrazine ring would be contained in its structure. Furthermore, in nuclear magnetic resonance (NMR) spectrum, signals at 0.99 (6H, doublet, J=6.5 Hz), 2.47 (1H, multiplet) and 2.89 ppm (2H,

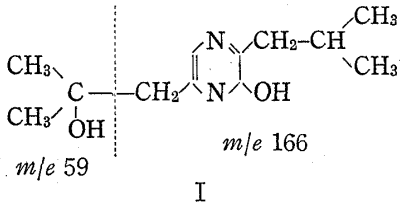
1) A part of this work was reported at the 92th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April, 1972.
2) Location: *Izumicho, Narashino-shi, Chiba.*
3) M. Yamazaki, Y. Maebayashi, and K. Miyaki, *Appl. Microbiol.*, **20**, 452 (1970).
4) G. Dunn, G.T. Newbold, and F.S. Spring, *J. Chem. Soc.*, **1949**, 2586.
5) J.C. MacDonald, R.G. Micetich, and R.H. Haskins, *Can. J. Microbiol.*, **10**, 90 (1964).

doublet, J=6.5 Hz) were observed, indicating the presence of an isobutyl group. On the other hand, two singlets at 1.37 (6H) and 2.75 ppm (2H) suggested an another presence of isobutyl side chain in which the carbon atom bearing two methyl groups might be substituted. A singlet of one proton on pyrazine ring was observed at 7.40 ppm also in the spectrum. In addition, the observation of the peaks of *m/e* 166 ($C_9H_{14}ON_2$) and 59 (base peak, C_3H_6OH) other than 224 (parent peak) and 123 ($C_6H_7ON_2$) in mass spectrometry supported a supposition that the hydroxyl was present at β-position of one of the side chain. The chemical shift in NMR of each protons on the isobutyl was quite similar to that of isobutyl attached at 3-position of pyrazine ring in flavacol:

Compound	Subtd. at	Chemical shift in $CDCl_3$ (ppm)		
I	3	0.99 (6H, d)	2.47 (1H, m)	2.89 (2H, d)
	6	1.37 (6H, s)		2.75 (2H, s)
Flavacol	3	1.03 (6H, d)	2.50 (1H, m)	2.92 (2H, d)
	6	0.85 (6H, d)	2.00 (1H, m)	2.35 (2H, d)

It would therefore be probable to assume that the substitution of the hydroxylated side chain on pyrazine ring should be on 6 but not 3. Conclusively the structure of newly isolated pyrazine metabolite was postulated as being I.

Besides above three metabolites, a red pigment, mp 129—133° (reaflets from aq. acetone) has been isolated from ethyl acetate extract of mycelia by silica gel column chromatography. By treatment of the pigment with 2N NaOH–MeOH, dark brown precipitates were obtained and subsequently determined as ferric hydroxide by coloring with ferrocyanide ion. From the filtrate, colorless needles of neoaspergillic acid, mp 123—125° were obtained and identified with authentic specimen by comparison of the spectral data and mixed fusion.



I

Of interest is that I is the first pyrazine compound having hydroxyl group on the β-carbon of side chain in contrast to that all of the hydroxyl on side chain of the fungal pyrazines having been known in attached to the α-carbon. As a plausible way of β-hydroxylation, a following route may be considered: α-hydroxylation→dehydration→re-hydroxylation on β-carbon (*via* epoxidation and reduction?).

Experimental

Microorganism——*Aspergillus ochraceus* WILH. IFM 4443 which was isolated in this laboratory from moldy rice and maintained on malt extract and Czapek agar containing 20% sucrose, was cultivated as previously reported.[3]

Isolation of I, Flavacol and Neoaspergillic Acid——Isolation of the pyrazines was carried out according to the procedure illustrated in Chart 1.

Flavacol (AO-2)——Colorless needles, mp 149—150° from ethyl acetate. UV λ_{max}^{EtOH} nm (ε) 229 (4290), 325 (4660). IR_{KBr} cm^{-1}: 3410, 3060, 2950, 1640, 1467, 1364, 1240, 955. NMR in $CDCl_3$ δ ppm 0.85 (6H, doublet, J=7.0), 1.03 (6H, doublet, J=7.0), 2.00 (1H, multiplet), 2.35 (2H, doublet, J=7.0), 2.50 (1H, multiplet), 2.92 (2H, doublet, J=7.0), 7.26 (1H, singlet), 13.20 (1H, broad singlet). Identified with authentic sample of flavacol.

Neoaspergillic Acid (AO-1′)——Colorless needles, mp 126—127.5° from ethanol. Reddish brown with $FeCl_3$. UV λ_{max}^{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 (3H, doublet, J=7.0), 0.94 (3H, doublet, J=7.0), 1.96—2144 (2H, multiplet), 2.67 (4H, triplet, J=7.0), 9.71 (1H, broad singlet). Identified with authentic sample of neoaspergillic acid.

Neoaspergillic Acid -Fe (AO-1)——Red leaflets, mp 129—133° from acetone–H_2O. Soluble in most organic solvent but insoluble in water. UV λ_{max}^{EtOH} nm 227, 315, 410. IR $_{KBr}$ cm^{-1}: 3440, 2955, 2860, 1583, 1523, 1488. Afforded AO-1′ and $Fe(OH)_3$ by disolving in 2N NaOH–MeOH (2:1).

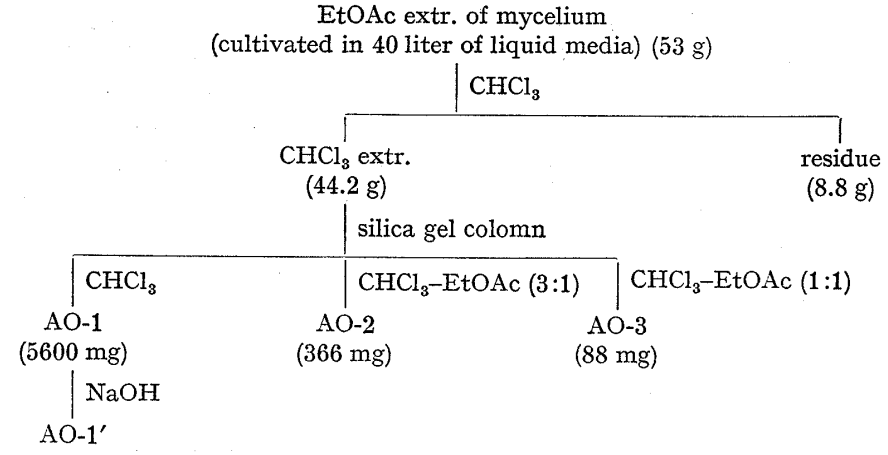


Chart 1

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).

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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).