

[Chem. Pharm. Bull.]
29(4)1005-1012(1981)]

**Metabolic Products of *Aspergillus terreus*. VI.¹⁾ Metabolites of the Strain
IFO 8835. (3).²⁾ The Isolation and Chemical
Structures of Colorless Metabolites**

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(Received September 30, 1980)

Seven colorless metabolites related to asterriquinones and one colorless compound of another type were isolated from *Aspergillus terreus* IFO 8835, and their chemical structures were determined.

Keywords—asterriquinones; *Aspergillus terreus*; IFO 8835; indolyl; indoline; nicotiny!

During the chromatographic separation of asterriquinones (AQ-A-1 through AQ-D),³⁾ a number of colorless compounds were also isolated from the mycelia of *Aspergillus terreus* var. *africanus* IFO 8835. This paper deals with these colorless metabolites.

The isolation methods were fundamentally the same as those reported in the previous paper,²⁾ but more careful and repeated chromatography was necessary for the isolation of these metabolites.

Compound I, mp 242–245°, $C_{34}H_{36}N_2O_4$, was eluted together with AQ-A-1, and it was isolated from the fraction slightly soluble in methanol.

Compound II, mp 206–208°, $C_{29}H_{28}N_2O_4$, was found in the fraction containing AQ-C-1, and was isolated from the fraction slightly soluble in benzene.

These two colorless compounds were gradually oxidized in the air, or immediately on being shaken with aqueous ferric chloride, to provide the corresponding purple quinones, AQ-A-1 and AQ-C-1, respectively. These quinones reverted to I and II upon reduction with sodium hydrosulfite. Thus, Compounds I and II were identified as the quinols of AQ-A-1 and AQ-C-1, respectively. The yields of I and II were increased by rapid treatment of the extract.

The color of the extraction solution from mycelia was light orange at first, but it changed to purple during prolonged extraction and chromatographic separation. These results suggested that “asterriquinones” existed as their quinol forms in the mycelia of IFO 8835, at least partially.

Three compounds of another types (III, IV, and V) were also isolated. Compound III, mp 158–159°, $C_{36}H_{40}N_2O_4$, was found in the fraction containing AQ-A-1. Compound IV, mp 202–204°, $C_{31}H_{32}N_2O_4$, was detected in the fraction containing AQ-A-3 and AQ-A-4, and Compound V, mp 226.5–228.5°, in the mixture of AQ-B-3 and AQ-B-4. These three compounds were sparingly soluble in cold methanol, and were isolated by crystallization from methanol.

Compounds III, IV, and V were positive to the Ehrlich reaction, and showed similar ultraviolet (UV) and infrared (IR) spectra. They had four methoxyl groups and dimethylallyl group (s). The dimethylallyl groups were classified as 1,1- or 3,3-dimethylallyl groups by proton nuclear magnetic resonance (PMR) spectroscopy. From these results, compounds III, IV, and V were proposed to be the dimethyl ethers of quinols of AQ-A-1, AQ-B-3, and AQ-C-2, respectively.

Compound III was obtained from AQ-A-1 by reducing with sodium hydrosulfite to the corresponding quinol (I) followed by methylation with diazomethane. Compounds IV and V

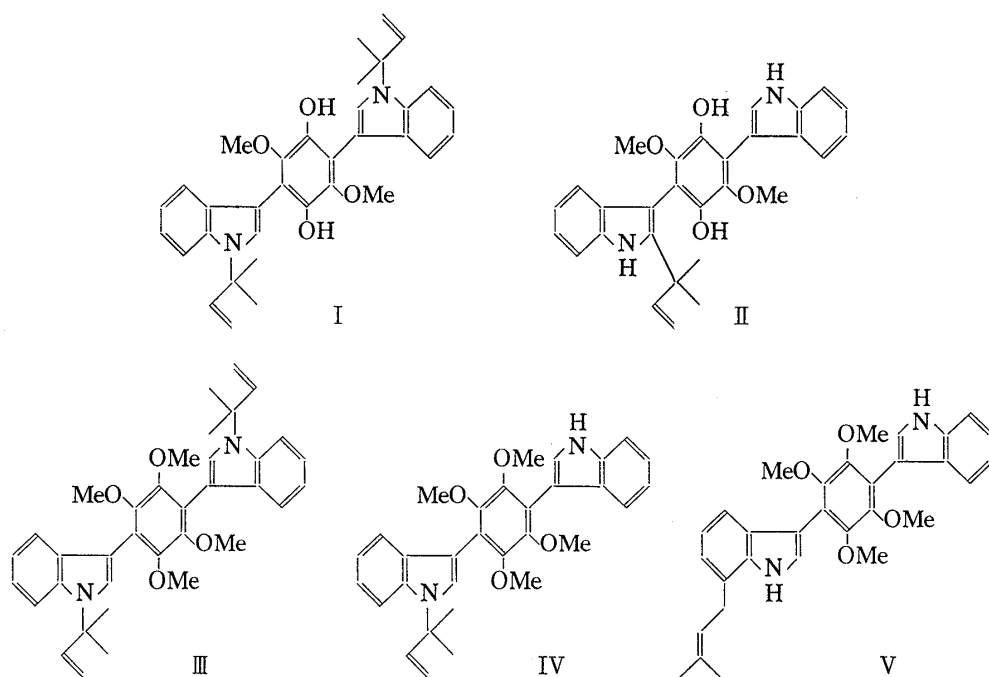


Fig. 1

were also obtained from AQ-B-3 and AQ-C-2, respectively, by the same method.

On the basis of these results, compounds III, IV, and V were identified as the dimethyl ethers of the quinols of AQ-A-1, AQ-B-3, and AQ-C-2, respectively.

Four other colorless metabolites (VI—IX) were newly isolated from the ether extract of the mycelia. After elution of all the pigments from a silica gel column with benzene-ethyl acetate (8:2), the ethyl acetate content was increased to 30% and then 50%. The pale yellow eluate with 30% ethyl acetate was concentrated and re-chromatographed on silica gel with benzene-ethyl acetate (8:2). The first pale yellow eluate was evaporated to dryness and the residue was purified from carbon tetrachloride as a slightly brown powder (VI), mp 134—138° (yield, 1.8 g from 18 l of culture medium). The following fraction gave compound VII, which was purified from benzene as a slightly brownish powder, mp 131—140° (unclear) (yield, 230 mg from 18 l of culture medium).

Two other compounds (VIII and IX) were isolated from the 50% ethyl acetate fraction. The eluate was evaporated to dryness and the residue was treated with methanol. The less soluble part was crystallized from benzene as colorless needles (VIII), mp 221—223° (yield, 200 mg from 18 l of culture medium). The soluble part was re-chromatographed on alumina with benzene-ethyl acetate (9:1), and the first fraction was crystallized from methanol as colorless prisms (IX), mp 142—143° (yield, 400 mg from 18 l of culture medium). VIII was also isolated from the next fraction.

The UV peaks (223, 284, and 291 nm) of VI and VII suggested the presence of indole rings, whereas VIII and IX showed different UV spectra.

Compound VI, $C_{25}H_{22}N_2O_5$, had optical activity. It was reduced with lithium aluminum hydride in boiling tetrahydrofuran to provide a colorless compound (X), mp 285—288°. This compound (X) had no optical activity, and the molecular formula, $C_{25}H_{22}N_2O_4$, suggested the loss of one oxygen atom in this reaction. Compound X was also obtained by treatment of VI with zinc powder in acetic acid.

Compound X had three methoxyl groups, two indole ring NH groups, and one phenolic hydroxyl group. It was soluble in sodium hydroxide, and was methylated with diazomethane to provide a methyl ether (XI), mp above 300°, $C_{26}H_{24}N_2O_4$. This methyl ether had four methoxyl groups, and symmetrical structure was suggested by the PMR spectrum. Thus,

XI was proposed to be the dimethyl ether of the quinol of AQ-D, and this was confirmed by reduction of AQ-D with sodium hydrosulfite followed by methylation with diazomethane. These results showed that VI had the same skeleton as AQ-D.

The PMR spectrum of VI showed three methoxyl groups and nine aromatic protons. The signal at δ 10.34 (1H) was assigned to the NH group of an indole ring. Another NH signal at δ 6.45 (d, $J=3.7$ Hz) was coupled with a methine proton at δ 6.22 (d, $J=3.7$ Hz), and the coupling was lost upon addition of deuterium oxide. Thus, it was suggested that one of the two indole rings in VI was modified in the pyrrole moiety.

Compound VI was insoluble in aqueous sodium hydroxide and was not methylated on treatment with diazomethane. The PMR signal at δ 5.30 (1H) was assigned to an alcoholic hydroxyl group (IR, 3550 cm^{-1} in CHCl_3).

These results suggested that the alcoholic hydroxyl group in VI was eliminated, and a new phenolic hydroxyl group was formed upon treatment with lithium aluminum hydroxide.

The last oxygen atom in VI was considered to be an ether one.

Compound VI gave a diacetate (XII), mp $227\text{--}228^\circ$, $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_7$, on acetylation with acetic anhydride in pyridine. From the IR spectrum, one of the acetyl groups was an alcoholic one (1730 and 1278 cm^{-1}), while the other was an N-acetyl group (1670 cm^{-1}) on the modified pyrrole moiety; the latter assignment was supported by the change of the doublet signal of the methine group (δ 6.22) to a singlet (δ 7.18). The PMR signal of the aromatic proton at

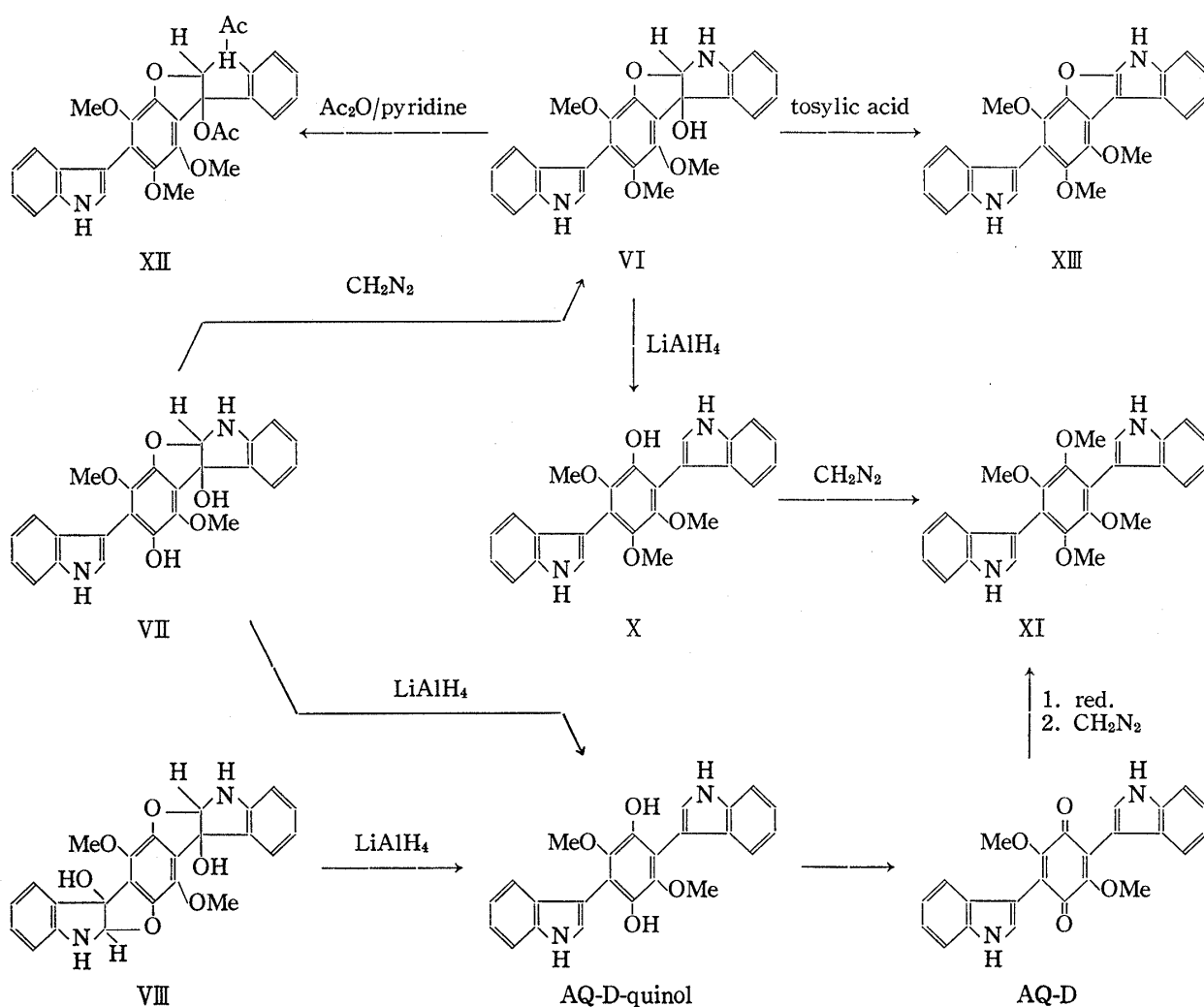


Fig. 2

position 7 in the modified indole ring was shifted downfield (δ 8.09) by this acetylation.⁴⁾ Two characteristic signals at δ 91.06 and 98.92 in the CMR spectrum of XII were assigned to aliphatic carbons carrying oxygen at positions 2 and 3 of the modified indole ring, respectively.

On the basis of these results, the presence of a 3-hydroxyindoline moiety in VI was proposed.

Compound VI was treated with *p*-toluenesulfonic acid to yield colorless prisms (XIII), mp 272—274°, $C_{25}H_{20}N_2O_4$. The molecular formula and PMR spectrum indicated the elimination of a water molecule from VI. In this reaction, the methine group (δ 6.22) was lost and the second indole NH (δ 11.19) appeared. Thus, the chemical structure of compound VI was determined to be as shown in Fig. 2.

Compound VII had a molecular formula of $C_{24}H_{20}N_2O_5$, and was optically active. This compound was presumed to have a structure closely related to that of VI in view of its physical properties. VII was soluble in aqueous sodium hydroxide, and had two methoxyl groups, one phenolic hydroxyl group (δ 6.71) and one alcoholic hydroxyl group (δ 5.32). Compound VII was methylated with diazomethane to provide a methyl ether which was identical with VI. VII gave AQ-D on treatment with lithium aluminum hydride.

Thus, the structure of VII was determined to be as shown in Fig. 2.

Compound VIII was easily crystallized from methanol or benzene as colorless needles. It had optical activity, and the molecular formula $C_{24}H_{20}N_2O_6$ was assigned. The PMR and CMR spectra suggested a symmetrical structure. The PMR spectrum showed two NH groups (δ 6.33, d, $J=3.7$ Hz), a methine group (δ 6.13, d, $J=3.7$ Hz), and a hydroxyl group (δ 5.17), as had been observed in the PMR spectrum of VI. The UV spectrum (235, 305, and 320 nm) suggested the presence of an indoline moiety⁵⁾ and the absence of an indole ring.

Compound VIII gave AQ-D on treatment with lithium aluminum hydride. The chemical structure and reactions of VIII are shown in Fig. 2.

Compound IX had the molecular formula $C_{24}H_{25}N_3O_7$ and showed the IR bands of an NH groups (3250 cm^{-1}), OCH_3 (2830 cm^{-1}), and three carbonyl groups (1635 , 1675 , and 1710 cm^{-1}). In the PMR spectrum, the presence of four methoxyl group was recognized (δ 3.75, 3.89, 3.92, and 3.94).

Compound IX was insoluble in cold sodium hydroxide, but it became soluble within a few minutes upon warming the solution on a water bath to give an acid (XIV), mp 227—228°, $C_{23}H_{21}N_3O_7$. Thus, one of the four methoxyl groups was assigned to a methyl ester (IR, 1710 cm^{-1}).

Nine aromatic protons (δ 7.28—9.17) were recognized in IX by analysis of the PMR spectrum. These protons could be classified into three groups. The protons of the first group (δ 8.85, 8.14, 7.64, and 7.28) were coupled to one another and suggested the presence of an *o*-substituted aromatic ring. Another four protons at lower field (δ 9.17, 8.75, 8.25, and 7.35) were located in another aromatic ring. The chemical shift and spin-spin couplings suggested the presence of a pyridine ring substituted with a CO group at position 3. The last proton (δ 7.33) was a singlet, and was located on the third aromatic ring.

On the basis of these results, IX was considered to contain three partial structures, as shown in Fig. 3. The presence of two $-NH-CO-$ groups was recognized in the PMR (NH, δ 9.55 and 12.40) and IR (CO, 1675 and 1635 cm^{-1}) spectra. Compound IX was thus assumed to be a tripeptide. The mass spectrum of IX also supported this assumption.

Compound IX was hydrolyzed with 1 *N* NaOH by heating at 100° for one hr, and the reaction mixture was extracted with ethyl acetate after being acidified with hydrochloric acid.

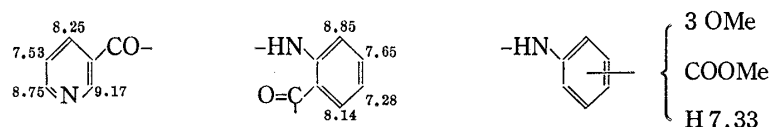


Fig. 3

The extract was crystallized from benzene to provide colorless prisms (XV), mp 178—180°. The aqueous layer was evaporated to dryness and the residue was extracted with ethanol. The ethanol extract gave nicotinic acid hydrochloride, mp 272° which was identified by IR spectroscopy.

Compound XV, $C_{17}H_{18}N_3O_6$, had one $-NH-CO-$ group (IR, 1648 cm^{-1} , PMR, δ 6.75) and was presumed to be a dipeptide. In the PMR spectrum, an NH_2 signal was observed, and all four aromatic protons of the first group were shifted to higher field.

Compound XV was further hydrolyzed by boiling with 2.5 N NaOH for 4 hr. The reaction mixture was fractionated by preparative thin layer chromatography (TLC). From the upper band, anthranilic acid, mp 144—145° was obtained, and another slightly yellow compound (XVI), mp 141—142°, was isolated from the lower band.

Compound XVI, $C_{10}H_{13}NO_5$, had three methoxyl groups, one NH_2 group, one $COOH$ group, and one aromatic ring proton (δ 7.16). Thus, XVI was presumed to be a trimethoxylantranilic acid. The chemical shift of the aromatic proton suggested the location of the proton at the *ortho*-position relative to the methoxyl and carboxyl groups, and *meta*-position relative to the amino group. The UV absorption (λ_{max}^{EtOH} , 250 nm) eliminated the *p*-aminobenzoic acid structure (280 nm), and the IR absorption of the CO group (1668 cm^{-1}) excluded the *m*-aminobenzoic acid structure (1635 cm^{-1}).

Thus, the structure of XVI was presumed to be 3,4,5-trimethoxylantranilic acid. This compound was reported by Meyer *et al.*⁶ as greenish needles of mp 136—137°, but this did

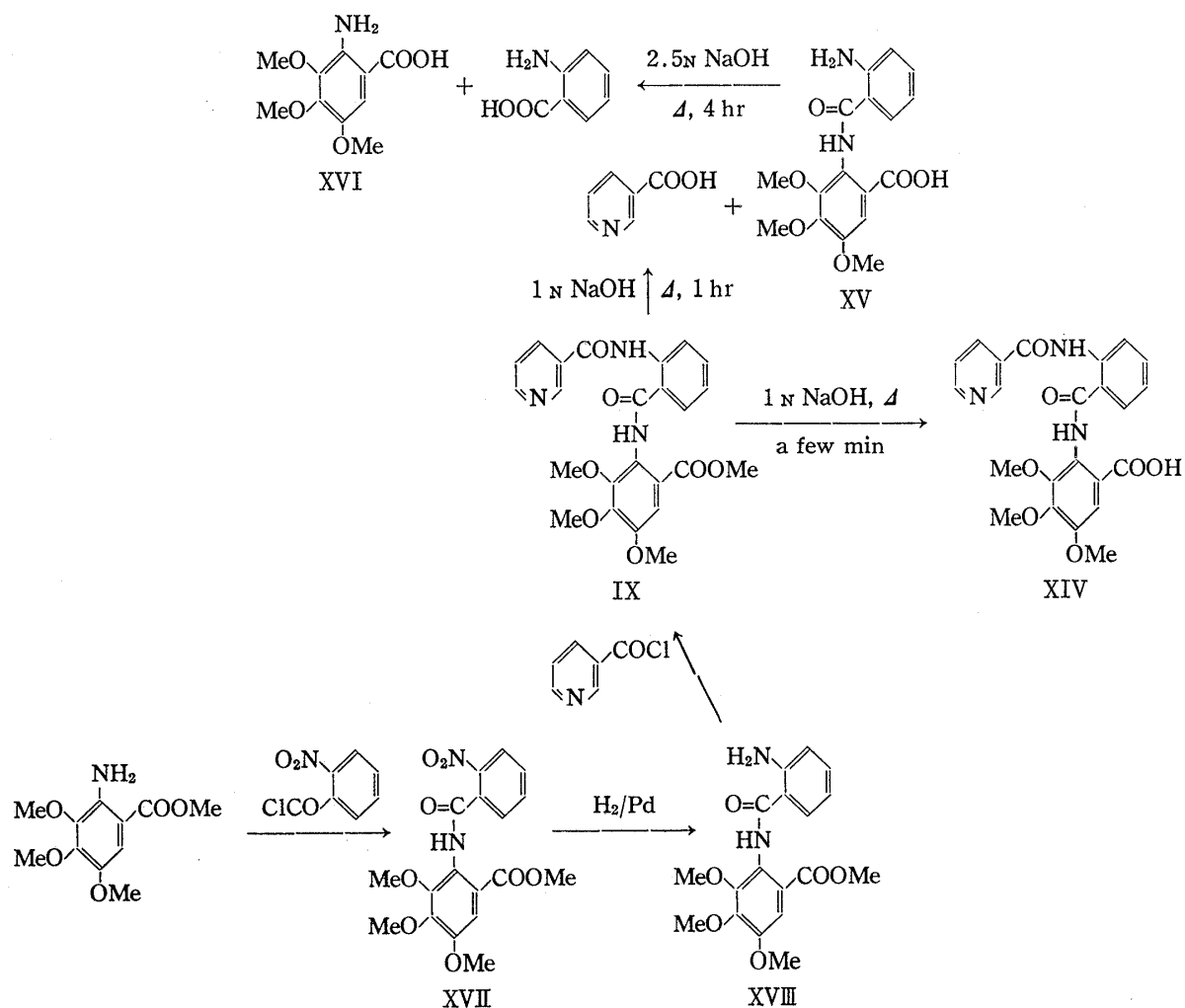


Fig. 4

not coincide with our data for XVI. Thus, XVI was synthesized from the trimethyl ether of gallic acid according to the literature,⁶⁾ and found to be identical with our product. The structure of IX was finally confirmed by total synthesis. The degradation and the synthesis of IX are summarized in Fig. 4.

Compound IX showed contractive activity for smooth muscles (bronchial and intestinal muscles) and cardinal muscle of guinea pigs, but had no anti-inflammatory activity.

Experimental⁷⁾

Compound VI—This was crystallized from CCl_4 as a slightly brown powder, mp 134–138°. *Anal.* Calcd for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_5$: C, 69.75; H, 5.15; N, 6.51. Found: C, 68.90; 70.26; H, 4.98; 5.46; N, 6.68; 6.51. MS m/e : 430 (M^+). $[\alpha]_{\text{D}}^{25} + 34.3^\circ$ ($c=0.11$, EtOH). PMR (acetone- d_6) δ : 3.31, 3.52, 4.08 (each 3H, s, OCH_3), 5.30 (1H, s, OH), 6.22 (1H, d, $J=3.7$ Hz, $-\text{CH}=\text{}$), 6.45 (1H, d, $J=3.7$ Hz, NH), 6.68–7.78 (9H, m, aromatic H), 10.34 (1H, s, NH).

Reduction of VI with LiAlH_4 (Formation of X)— LiAlH_4 (200 mg) was added to a solution of VI (70 mg) in tetrahydrofuran (5 ml), and the mixture was refluxed for 2.5 hr. After cooling, the reaction mixture was acidified with HCl and extracted with AcOEt. The solvent was evaporated off and the residue was chromatographed on silica gel (benzene–AcOEt, 5:1). The eluate was evaporated to dryness and the residue was crystallized from EtOH as colorless prisms (X), mp 285–288° (dec.) (yield, 31 mg). *Anal.* Calcd for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}_4$: C, 72.45; H, 5.35; N, 6.76. Found: C, 72.37; H, 5.22; N, 6.81. MS m/e : 414 (M^+).

Methylation of X with CH_3N_2 —Excess ethereal CH_3N_2 was added to a solution of X (50 mg) in tetrahydrofuran (3 ml) and MeOH (1.5 ml), and the mixture was kept for 24 hr. The solvent was evaporated off and the residue was crystallized from MeOH as colorless prisms (XI), mp above 300° (dec.) (yield, 43 mg). It was identical with the dimethylether of the quinol of AQ-D.

Acetylation of VI—A solution of VI (50 mg) in pyridine (0.5 ml) was treated with Ac_2O (5 ml) overnight at room temperature. The reaction mixture was poured into ice-water, and the resulting precipitate was collected. The mother liquor was extracted with Et_2O . The ether solution was washed with alkali and evaporated to dryness. The residue was combined with the precipitate and chromatographed on silica gel⁸⁾ (benzene–AcOEt, 4:1). The main fraction was crystallized from MeOH as colorless needles (XII) (46 mg), mp 227–228°. *Anal.* Calcd for $\text{C}_{29}\text{H}_{26}\text{N}_2\text{O}_7$: C, 67.69; H, 5.09; N, 5.45. Found: C, 67.48; H, 5.08; N, 5.34. MS m/e : 514 (M^+). $[\alpha]_{\text{D}}^{25} + 76^\circ$ ($c=0.11$, dioxane).

Dehydration of VI (Formation of XIII)—i) A solution of VI (300 mg) and *p*-toluenesulfonic acid (100 mg) in benzene (40 ml) was warmed at 75° for 10 min. The reaction mixture was washed with 10% NaHCO_3 and then H_2O . The solvent was evaporated off and the residue was chromatographed on silica gel (benzene–AcOEt, 9:1). The main fraction was evaporated to dryness and the residue was crystallized from MeOH as colorless prisms, mp 272–274° (yield, 180 mg). *Anal.* Calcd for $\text{C}_{25}\text{H}_{20}\text{N}_2\text{O}_4$: C, 72.80; H, 4.89; N, 6.79. Found: C, 73.04; H, 4.86; N, 6.55. MS m/e : 412 (M^+).

ii) SOCl_2 (0.4 ml) was added dropwise to a solution of VI (100 mg) in pyridine (5 ml) under ice cooling. After 5 min, the reaction mixture was evaporated to dryness and the residue was purified by silica gel chromatography as described above to obtain XIII (23 mg).

Compound VII—The effluent was treated with 0.1 N NaOH to remove contaminating VI, and the acidic part was crystallized from benzene as a slightly brownish powder, mp 131–140°. *Anal.* Calcd for $\text{C}_{24}\text{H}_{20}\text{NO}_5 \cdot 1/2\text{C}_6\text{H}_6$: C, 71.21; H, 5.05; N, 6.15. Found: C, 71.25; H, 5.11; N, 5.86. $[\alpha]_{\text{D}}^{25} + 51.2^\circ$ ($c=0.13$, dioxane).

Compound VII (50 mg) in MeOH (5 ml) was methylated with excess ethereal CH_3N_2 . The mixture was allowed to stand overnight, then the solvent was evaporated off and the residue was crystallized from CCl_4 as a slightly brownish powder, mp 130–134°. It was identical with VI.

VII (50 mg) was treated with LiAlH_4 under the conditions described for VI. The product was identical with AQ-D (yield, 20 mg).

Compound VIII—After elution of VII, a mixture of VIII and IX was eluted with benzene–AcOEt (1:1). The eluate was evaporated to dryness and the residue was treated with MeOH. Compound VIII was isolated as the less soluble compound, and crystallized from MeOH as colorless needles, mp 221–222°. *Anal.* Calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_6 \cdot \text{H}_2\text{O}$: C, 63.99; H, 4.92; N, 6.22. Found: C, 64.27; 64.37; H, 4.95; 4.73; N, 6.55, 6.22. The anhydrous compound was obtained by crystallization from benzene as colorless needles, mp 221–223°. *Anal.* Calcd for $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_6$: C, 66.66; H, 4.66; N, 6.48. Found: C, 66.65; H, 4.58; N, 6.31. MS m/e : 432 (M^+). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 214, 237(sh), 305, 320(sh), $[\alpha]_{\text{D}}^{25} + 39^\circ$ ($c=0.11$, dioxane).

VIII (50 mg) was treated with LiAlH_4 (100 mg) in tetrahydrofuran (5 ml) under reflux for 30 min. After cooling, the reaction mixture was diluted with H_2O , acidified, and extracted with AcOEt. The solvent was evaporated off and the residue was crystallized from acetone as dark-purple prisms, mp above 300° (yield, 23 mg). This compound was identical with AQ-D, which was also obtained from VII by LiAlH_4 treatment.

Compound IX—The MeOH mother liquor after removal of VIII was evaporated to dryness and the

residue was chromatographed on alumina with benzene-AcOEt (9:1). The first eluate was evaporated to dryness and the residue was crystallized from MeOH as colorless needles, mp 141–143°. *Anal.* Calcd for $C_{24}H_{23}N_3O_7$: C, 61.93; H, 4.98; N, 9.03. Found: C, 62.07; H, 4.95; N, 9.07. MS m/e : 465 (M^+). UV λ_{max}^{EtOH} nm: 216, 246, 260, 306. IR ν_{max}^{KBr} cm^{-1} : 3250 (NH), 2830 (OCH_3), 1710, 1675, 1635 (C=O). PMR data are shown in Fig. 3. MS m/e : 465 (M^+), 433, 324, 241, 106, etc.

Compound XIV—Compound IX (100 mg) was suspended in 1 N NaOH (5 ml), and warmed for a few min on a water bath. The solution was acidified with HCl and the resulting precipitate was collected and crystallized from MeOH as colorless prisms (XIV), mp 227–228°. *Anal.* Calcd for $C_{23}H_{21}N_3O_7$: C, 61.19; H, 4.69; N, 9.31. Found: C, 60.96; H, 4.56; N, 9.30. MS m/e : 451 (M^+).

Hydrolysis of IX to XV and Nicotinic Acid—Compound IX (500 mg) was added to 1 N NaOH (20 ml) and heated on a water bath for 1 hr. After cooling, the solution was acidified with HCl, and extracted with AcOEt. The solvent was evaporated off and the residue was crystallized from benzene to give colorless prisms (XV), mp 178–180° (yield, 200 mg). *Anal.* Calcd for $C_{17}H_{15}N_2O_6$: C, 58.95; H, 5.24; N, 8.09. Found: C, 59.13; H, 5.05; N, 8.10. MS m/e : 346 (M^+), 227, 210, 120, etc.

The aqueous layer was concentrated *in vacuo*, and the residue was extracted with EtOH. The soluble part was crystallized from EtOH as colorless prisms, mp 270–272°; this material was identified with authentic nicotinic acid hydrochloride (yield, 32 mg). *Anal.* Calcd for $C_6H_5NO_2 \cdot HCl$: C, 45.14; H, 3.76; N, 8.78. Found: C, 44.72; H, 3.63; N, 8.70.

Hydrolysis of XV—A solution of XV (100 mg) in 10% NaOH (16 ml) was heated on a water bath for 4 hr under an N_2 atmosphere. The reaction mixture was adjusted to pH 4.5 and extracted with AcOEt. The solvent was evaporated off and the residue was extracted with boiling ligroin to remove the starting material. The ligroin solution was concentrated, and the residue was purified by preparative TLC with benzene-AcOEt (2:1). From the upper band, anthranilic acid, mp 144–145° was obtained as colorless prisms from benzene. It was identified by IR spectral comparison and mixed melting point determination. From the lower band of the TLC, colorless plates (XVI), mp 141–143°, were obtained by crystallization from benzene-petr. benzin mixture. *Anal.* Calcd for $C_{10}H_{13}NO_5$: C, 52.86; H, 5.77; N, 6.17. Found: C, 53.17; H, 5.76; N, 5.88. MS m/e : 227 (M^+). UV λ_{max}^{EtOH} nm: 223, 250, 339. IR ν_{max}^{KBr} cm^{-1} : 3480, 3380, 2550 (COOH), 1668 (C=O). PMR ($CDCl_3$) δ : 3.77, 3.83, 3.93 (each 3H, OCH_3), 7.15 (1H, s, aromatic H), 7.83 (3H, bs, NH_2 and COOH).

Synthesis of 3,4,5-Trimethoxyanthranilic Acid (XVI)—According to the literature,⁶⁾ gallic acid was methylated to provide the trimethyl ether. It was brominated, and the bromo derivative, mp 150°, was treated with NH_4OH and Cu powder. The resulting amino compound (total yield, 57%), mp 141–142°, was identical with XVI. This compound was methylated with CH_2N_2 , and was used for the following synthesis without purification.

N-(*o*-Nitrobenzoyl-3,4,5-Trimethoxy)anthranilic Acid Methyl Ester (XVII)—A solution of *o*-nitrobenzoyl chloride (1.9 g) in $CHCl_3$ (20 ml) was added to a solution of the methyl ester of XVI (2.45 g) in $CHCl_3$ (30 ml), and the mixture was gently warmed on a water bath for 1.5 hr. During the reaction, a few drops of pyridine were added occasionally to neutralize the liberated HCl. After cooling, the reaction mixture was washed with aq. HCl, $NaHCO_3$, and H_2O successively. The solvent was evaporated off and the residue was treated with boiling petr. ether to remove unchanged anthranilate. The residue was crystallized from MeOH as slightly yellow needles, mp 148.5–150° (yield, 3.2 g, 82%). *Anal.* Calcd for $C_{18}H_{18}N_2O_8$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.36; H, 4.54; N, 7.34. MS m/e : 390 (M^+).

Catalytic Reduction of Nitro Compound (XVII)—XVII (6 g) was dissolved in MeOH (250 ml), and hydrogenated with 10% Pd-C (0.5 g). After saturation with H_2 , the catalyst was filtrated off and the filtrate was evaporated to dryness. The residue was purified by chromatography or repeated crystallization from MeOH to provide colorless needles (XVIII) (4.5 g), mp 139–141°. This product was identical with the methyl ester of XV. *Anal.* Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 60.05; H, 5.59; N, 7.49. MS m/e : 360 (M^+).

Synthesis of IX—Nicotinic acid chloride (prepared from 400 mg of nicotinic acid by treatment with $SOCl_2$) in $CHCl_3$ (15 ml) and pyridine (0.26 ml) was added to a solution of XVIII (1.08 g) in $CHCl_3$ (30 ml), and the mixture was warmed on a water bath for 30 min with occasional addition of pyridine (total, 1.0 ml). After cooling, the reaction mixture was washed with H_2O , 10% $NaHCO_3$, and H_2O successively. The solvent was evaporated off, then the residue was washed with ether, and purified by repeated crystallization from MeOH as colorless needles, mp 142–143°. This compound was identical with the natural compound IX.

Acknowledgement We are grateful to the Institute for Fermentation, Osaka, for the gift of the IFO strain. Elementary analyses, and NMR and mass spectra were measured by Mr. Y. Itatani, and Misses Y. Arano and K. Ohata, and a part of this work was performed by Misses R. Okabe and M. Hanano, to whom our thanks are due. We thank Dr. S. Nagai, Hokuriku University, for his advice on peptide synthesis.

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- 7) All melting points are uncorrected.
- 8) Silica gel (Kanto, for chromatography) was used throughout this work.