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Metabolic Products of *Aspergillus terreus*. IV.[1] Metabolites of the Strain IFO 8835. (2). The Isolation and Chemical Structure of Indolyl Benzoquinone Pigments

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Eleven kinds of bisindolyl-dimethoxyl-*p*-benzoquinones, "asterriquinones A-1, A-2, A-3, A-4, B-1, B-2, B-3, B-4, C-1, C-2, and D" were isolated from *Aspergillus terreus* IFO 8835, and their structures were determined. Asterriquinone D had the basic structure, and the others were substituted with dimethylallyl group(s) in the indole ring.

Keywords——asterriquinone; *Aspergillus terreus*; IFO 8835; indolyl; benzoquinone; dimethylallyl

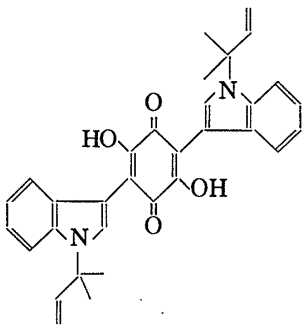
In the previous paper,[2] we reported the isolation of a tryptophan-derived purple benzoquinone named asterriquinone (I) from *Aspergillus terreus* IFO 6123. The antitumor activity of this compound was also demonstrated.[3]

In the course of the investigation of analogous antitumor compounds, we found 11 kinds of indolyl benzoquinones in *Aspergillus terreus* var. *africanus* IFO 8835, together with a butyrolactone derivative (II).[1]

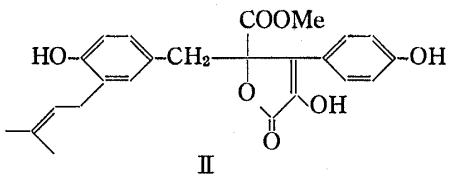
This paper deals with these new indolyl benzoquinones related to asterriquinone (I). We wish to propose the general name "asterriquinone" for these pigments: asterriquinones A-1, A-2, A-3, A-4, B-1, B-2, B-3, B-4, C-1, C-2, and D (abbreviated as AQ–A-1, AQ–A-2 and so on, or simply as A-1, A-2 and so on).

The fungus IFO 8835 was cultivated on a malt extract medium as reported in the preceding paper,[1] but the content of polypeptone was increased in order to improve the production of these pigments.

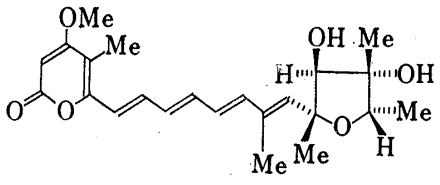
The dried mycelia were extracted with petroleum ether, and then ether. The petr. ether extract was treated with methanol, and the soluble part was chromatographed on silica gel after removal of the less soluble ergosterol. By elution with benzene, four pigments, AQ–A-1, A-2, A-3, and A-4, were isolated.



asterriquinone (I)



II



citreoviridin (III)

Fig. 1

The ether extract was washed with aqueous sodium carbonate and kept overnight. The yellow precipitate, mp 105—107° was identified as citreoviridin (III).[4] After removal of III, the ether extract was roughly separated into four groups, AQ–A, –B, –C, and –D, by silica gel chromatography with a mixture of benzene–ethyl acetate. Each group of pigments was further chromatographed on an alumina column with the same solvent system, and the separated pigments were purified by recrystallization. By this procedure, pigments of the A-group as well as B-1, B-2, B-3, B-4, C-1, C-2 and D were isolated. The procedure is sum-

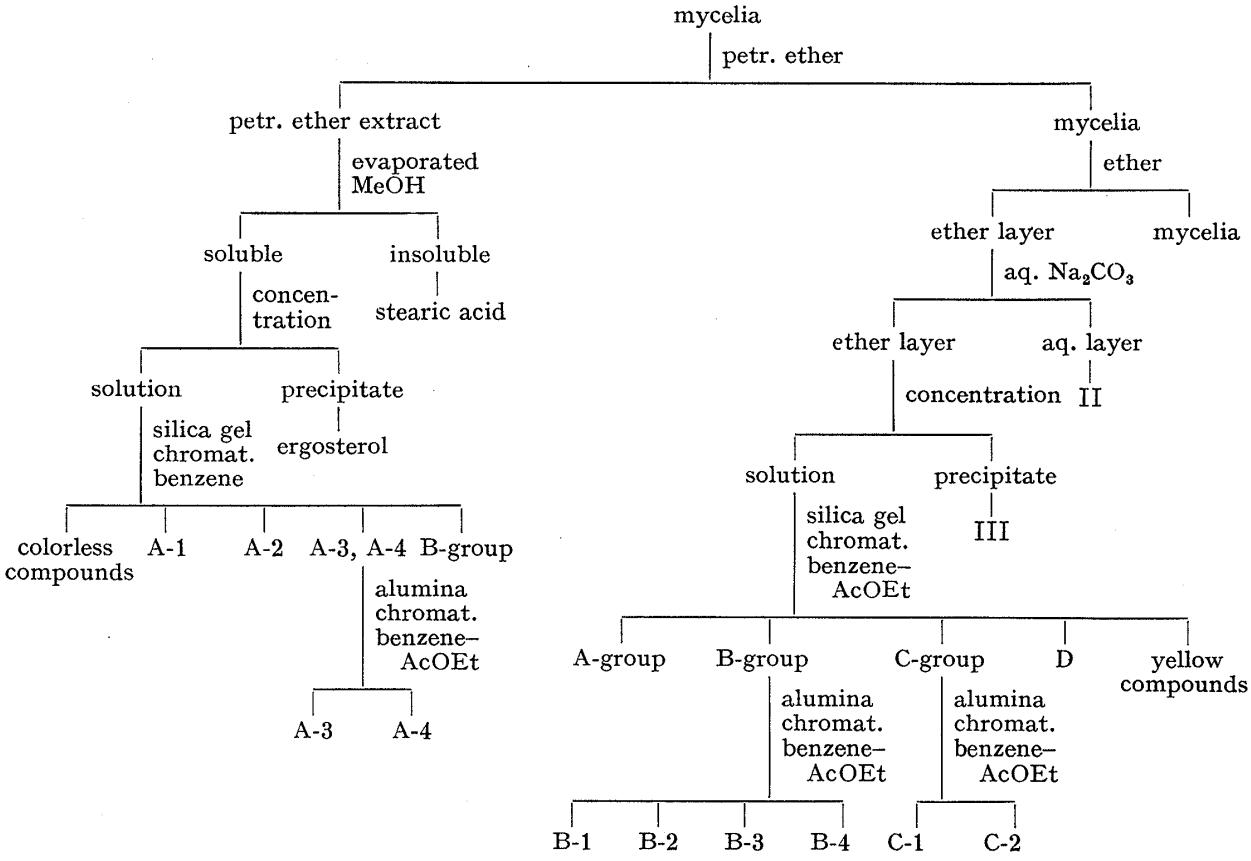


Fig. 2. Isolation Procedure for Asterriquinones

Table I. List of Asterriquinones

	mp	Formula (M^+ *m/e*)	Number of C_5H_9 groups		UV λ_{max}^{EtOH} nm
AQ–A-1	167—168°	$C_{34}H_{34}N_2O_4$ (534)	2	—	295, 299, 490
AQ–A-2	189—192°	$C_{39}H_{42}N_2O_4$ (602)	1	2	283, 292, 374, 505
AQ–A-3	112—114°	$C_{34}H_{34}N_2O_4$ (534)	2	—	284, 292, 376, 516
AQ–A-4	252—253°	$C_{39}H_{42}N_2O_4$ (602)	2	1	284, 291, 390, 497
AQ–B-1	208—209°	$C_{34}H_{34}N_2O_4$ (534)	1	1	285, 292, 382, 522
AQ–B-2	199—200°	$C_{34}H_{34}N_2O_4$ (534)	1	1	283, 292, 372, 518
AQ–B-3	148—150°	$C_{29}H_{26}N_2O_4$ (466)	1	—	284, 290, 484
AQ–B-4	>300°	$C_{34}H_{34}N_2O_4$ (534)	2	—	284, 292, 385, 502
AQ–C-1	213—214°	$C_{29}H_{26}N_2O_4$ (466)	1	—	283, 291, 367, 510
AQ–C-2	202—203°	$C_{29}H_{26}N_2O_4$ (466)	—	1	285, 290, 485
AQ–D	>300°	$C_{24}H_{18}N_2O_4$ (398)	—	—	284, 290, 488
AQ (I)	218—220° (dec.)	$C_{32}H_{30}N_2O_4$ (506)	2	—	298, 508

marized in Fig. 2.

The properties of these asterriquinones are summarized in Table I.

All these pigments contained *p*-benzoquinone moieties which were substituted with two indolyl and two methoxyl groups. They had almost identical ultraviolet (UV) spectra, as shown in Table I, and had no optical activity. The presence of two types of dimethylallyl groups (C_5H_9) was observed in the proton nuclear magnetic resonance (PMR) spectra, *viz.* 3,3-dimethylallyl and 1,1-dimethylallyl groups. The number of groups was easily determined from the mass and PMR spectra.

The difference of molecular weight between each pigment was 68 ($=C_5H_8$), so it was considered that these pigments were products which differed in the number, type, and substitution positions of dimethylallyl groups. In detail, N-substituted 1,1-dimethylallyl group was distinguished from C-substituted ones by the chemical shifts of the methyl groups (δ 1.75—1.86 for N-substituted, and δ 1.37—1.54 for C-substituted).

For the determination of the chemical structures of these pigments, oxidative cleavage of the benzoquinone ring with hydrogen peroxide was very useful, as reported in the case of asterriquinone (I).[2)]

In the oxidation reactions, the presence of methoxyl groups on the benzoquinone ring caused low yields of the products, and the double bond in dimethylallyl groups underwent various side reactions. Therefore, the methoxyl groups were demethylated with hydrochloric acid or potassium hydroxide after or before hydrogenation of double bonds in the side chains. Demethylation was also necessary for the investigation of antitumor activity, because free hydroxyl groups on the quinone ring seemed essential for the biological activity. Demethylation of these pigments will be reported in detail in the next paper (Part V).

Asterriquinone A-1 (AQ–A-1), obtained as reddish-purple needles from methanol, had a symmetrical structure as expected from the PMR spectrum. The difference between AQ–A-1 and asterriquinone (I) was the presence of methoxyl groups in the former, and AQ–A-1 was easily identified as the dimethyl ether of I. Thus, I gave AQ–A-1 upon methylation with diazomethane, and demethylation of AQ–A-1 gave I.

Asterriquinone D (AQ–D), obtained as a purple powder from acetone, had no dimethylallyl group, which suggested that AQ–D was the fundamental compound of the asterriquinone groups. Upon demethylation and successive degradation with hydrogen peroxide in an alkaline medium, AQ–D gave indole-3-carboxylic acid (IV), mp 212—214°, (yield, 35%). From these results, the structure of AQ–D was determined to be as shown in Fig. 4.

Asterriquinone C-1 (AQ–C-1), obtained as purple needles from benzene, was considered to be the mono-C-substituted 1,1-dimethylallyl (CH_3, δ 1.46) derivative of AQ–D, and the substituted position was proposed to be position 2 of the indole ring on the basis of the PMR spectrum. The structure was finally confirmed by oxidative degradation. AQ–C-1 was catalytically hydrogenated to *tert*-pentyl quinol, mp 206—208°, and was oxidized to the corresponding quinone (AQ–HC-1), mp 211—212°, with ferric chloride. AQ–HC-1 was demethylated with hydrochloric acid or potassium hydroxide to demethyl AQ–HC-1, mp 240—242°. This compound was oxidized with hydrogen peroxide in an alkaline or acetic acid medium. Under alkaline conditions, indole-3-carboxylic acid (IV) was obtained, and in the acidic solution, N-(2,2-dimethylbutyroyl)anthranilic acid (V), mp 137—138°, $C_{13}H_{17}NO_3$, was obtained, which was hydrolyzed to anthranilic acid, mp 144°. The reactions are shown in Fig. 3. From these results, the chemical structure of AQ–C-1 was determined to be as shown in Fig. 4.

Demethyl AQ–C-1, mp 242—244°, which was obtained by demethylation of AQ–C-1, gave a dilactone derivative (VI) as orange needles, mp above 300°, $C_{27}H_{20}N_2O_4$ (infrared (IR) spectrum, 1800, 1756 cm^{-1}) on oxidation with dimethylsulfoxide in acetic anhydride.[5)] This reaction showed the presence of a 2,5-dihydroxy-*p*-benzoquinone moiety in AQ–C-1.

Asterriquinone C-2 (AQ–C-2), obtained as purple needles from methanol, had the same formula as AQ–C-1, but the substituted group was a 3,3-dimethylallyl group. AQ–C-2 was

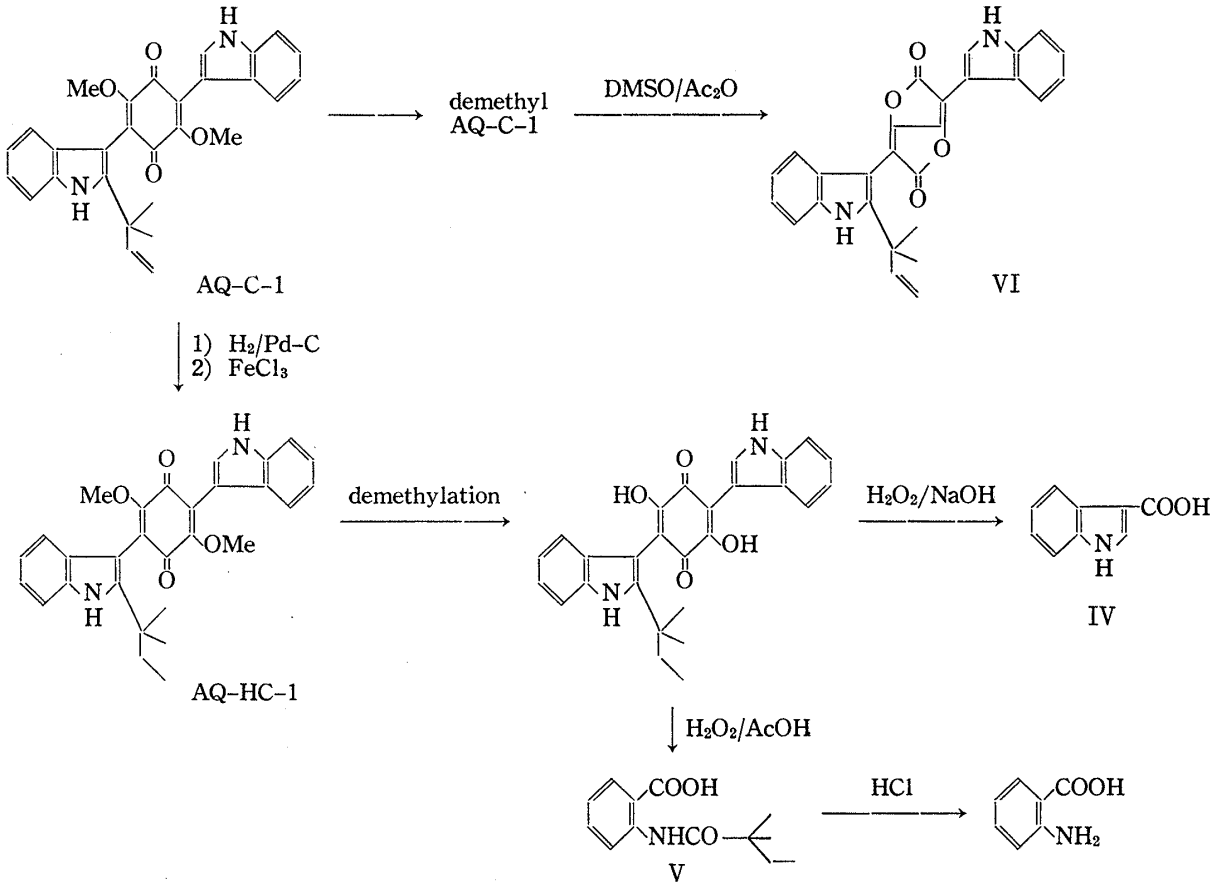


Fig. 3

catalytically hydrogenated, re-oxidized to dihydro-quinone (AQ–HC-2), mp 242—244°, and demethylated to demethyl AQ–HC-2, mp 262—265°. The demethyl AQ–HC-2 was oxidized with hydrogen peroxide in an alkaline medium to obtain indole-3-carboxylic acid (IV) and 7-isopentylindole-3-carboxylic acid (VII), mp 195—196°, $C_{14}H_{17}NO_2$.

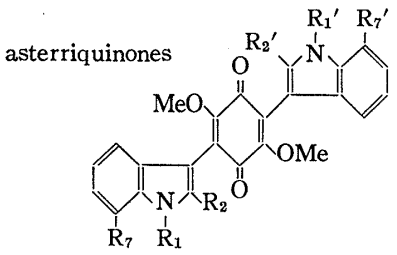
Demethyl AQ–HC-2 also gave 3-isopentylanthranilic acid (VIII), mp 123—124°, on oxidation with hydrogen peroxide in acetic acid. VIII was decarboxylated by heating at 210° to *o*-isopentylaniline (IX). This compound was acetylated to *o*-isopentylacetanilide,[6] mp 98—99°. From these results, the chemical structure of AQ–C-2 was determined to be as shown in Fig. 4.

Asterriquinone B-1 (AQ–B-1), obtained as deep purple prisms from benzene, had two dimethylallyl groups of different types. AQ–B-1 was catalytically hydrogenated and re-oxidized to the corresponding quinone, AQ–HB-1, mp 255—257°. It was demethylated and the demethyl compound, mp 243—244° was decomposed with hydrogen peroxide in acetic acid medium. The products were separated into three fractions by preparative thin-layer chromatography (TLC) (acid-washed silica gel, *n*-hexane–AcOEt, 1: 1). From the upper band of the TLC, colorless needles, mp 137—138° were obtained and found to be identical with authentic N-(2,2-dimethylbutyroyl)anthranilic acid (V). The middle band gave 7-isopentylindole-3-carboxylic acid (VII) as colorless plates, mp 195—196°; this product had already been obtained from AQ–HC-2. 3-Isopentylanthranilic acid (VIII) was obtained from the lowest band. From these results, the chemical structure of AQ–B-1 was determined to be as shown in Fig. 4.

Asterriquinone B-2 (AQ–B-2), obtained as deep purple prisms from benzene, had two different types of dimethylallyl groups like AQ–B-1. AQ–B-2 was hydrogenated, re-oxidized to the corresponding quinone (AQ–HB-2), mp 196—197°, and demethylated. The demethyl

AQ–HB-2, mp 212—214°, was treated with hydrogen peroxide. Indole-3-carboxylic acid (IV) was the only product of the reaction in an alkaline medium, and a material obtained as colorless needles, mp 116—117°, was the only product in the acetic acid medium. The latter, $C_{18}H_{27}NO_3$ was identified as 3-isopentyl-N-(2,2-dimethylbutyroyl)anthranilic acid (XI) which gave 3-isopentylanthranilic acid (VIII) on hydrolysis. From these results, the chemical structure of AQ–B-2 was determined to be as shown in Fig. 4.

Asterriquinone B-3 (AQ–B-3), obtained as a reddish-purple powder from cyclohexane, was judged to be a mono-N-1,1-dimethylallyl derivative of AQ–D on the basis of the PMR spectrum (CH_3, δ 1.83). AQ–B-3 was hydrogenated and re-oxidized to the corresponding quinone (AQ–HB-3), mp 80—82°. It was demethylated, and the demethyl AQ–HB-3, mp 191—193°, was oxidized with hydrogen peroxide in an alkaline medium. The products were indole-3-carboxylic acid (IV) and a material obtained as colorless needless, mp 153°, which was identified as N-*tert*-pentylindole-3-carboxylic acid (XII). XII had been obtained from the tetrahydro derivative of asterriquinone (I) by oxidation with hydrogen peroxide in an alkaline medium. Thus, the chemical structure of AQ–B-3 was determined to be as shown in Fig. 4.



	Positions of prenyl groups in asterriquinones			Positions of prenyl groups in asterriquinones	
AQ–A-1	R_1, R_1'	—	AQ–B-3	R_1	—
AQ–A-2	R_2	R_7, R_7'	AQ–B-4	R_2, R_2'	—
AQ–A-3	R_1, R_2'	—	AQ–C-1	R_2	—
AQ–A-4	R_2, R_2'	R_7	AQ–C-2	—	R_7
AQ–B-1	R_2	R_7'	AQ–D	—	—
AQ–B-2	R_2	R_7			

Fig. 4. Structures of Asterriquinones

Asterriquinone B-4 (AQ–B-4), obtained as reddish-purple plates from methanol, had two C-substituted 1,1-dimethylallyl groups as judged from its PMR spectrum (CH_3, δ 1.48). The PMR signal of the NH proton on the indole ring was a singlet, and the Ehrlich reaction was negative. Thus, the substituted position of the dimethylallyl group was proposed to be the 2 position of each indole ring.

The structure of AQ–B-4 was determined by isolation of V from demethyl AQ–HB-4 on treatment with hydrogen peroxide in acetic acid medium. The yield of V in this reaction (29%) was almost twice that in the case of demethyl AQ–HC-1.

Asterriquinone A-2 (AQ–A-2), obtained as deep purple prisms from benzene, had three dimethylallyl groups. Two of them were 3,3-dimethylallyl groups and the other was a C-substituted 1,1-dimethylallyl group. The structure was determined by the following degradation. Hexahydro AQ–A-2 (AQ–HA-2), mp 221—222°, was demethylated to demethyl AQ–HA-2, mp 190—192°. The demethyl compound was oxidized with hydrogen peroxide in an alkaline medium to obtain 7-isopentylindole-3-carboxylic acid (VII), mp 195—196°, together

with 3-isopentylanthranilic acid (VIII), mp 123—124°. When the demethyl AQ-HA-2 was treated with hydrogen peroxide in acetic acid, 3-isopentyl-N-(2,2-dimethylbutyroyl)anthranilic acid (XI), mp 116—117°, was obtained as in the case of demethyl AQ-HB-2, together with colorless plates (XIII), mp 175—177.5°, $C_{13}H_{15}NO_3$. The latter compound (XIII) had three vicinal aromatic protons and an isopentyl group (from the PMR spectrum). The presence of NH and anhydride groups (1795, 1730 cm^{-1}) was also suggested by the IR spectrum. The UV spectrum of XIII was very similar to that of isatonic acid anhydride,[7] so XIII was identified as 7-isopentylisatonic acid anhydride. The reactions of AQ-A-2 were summarized in Fig. 5.

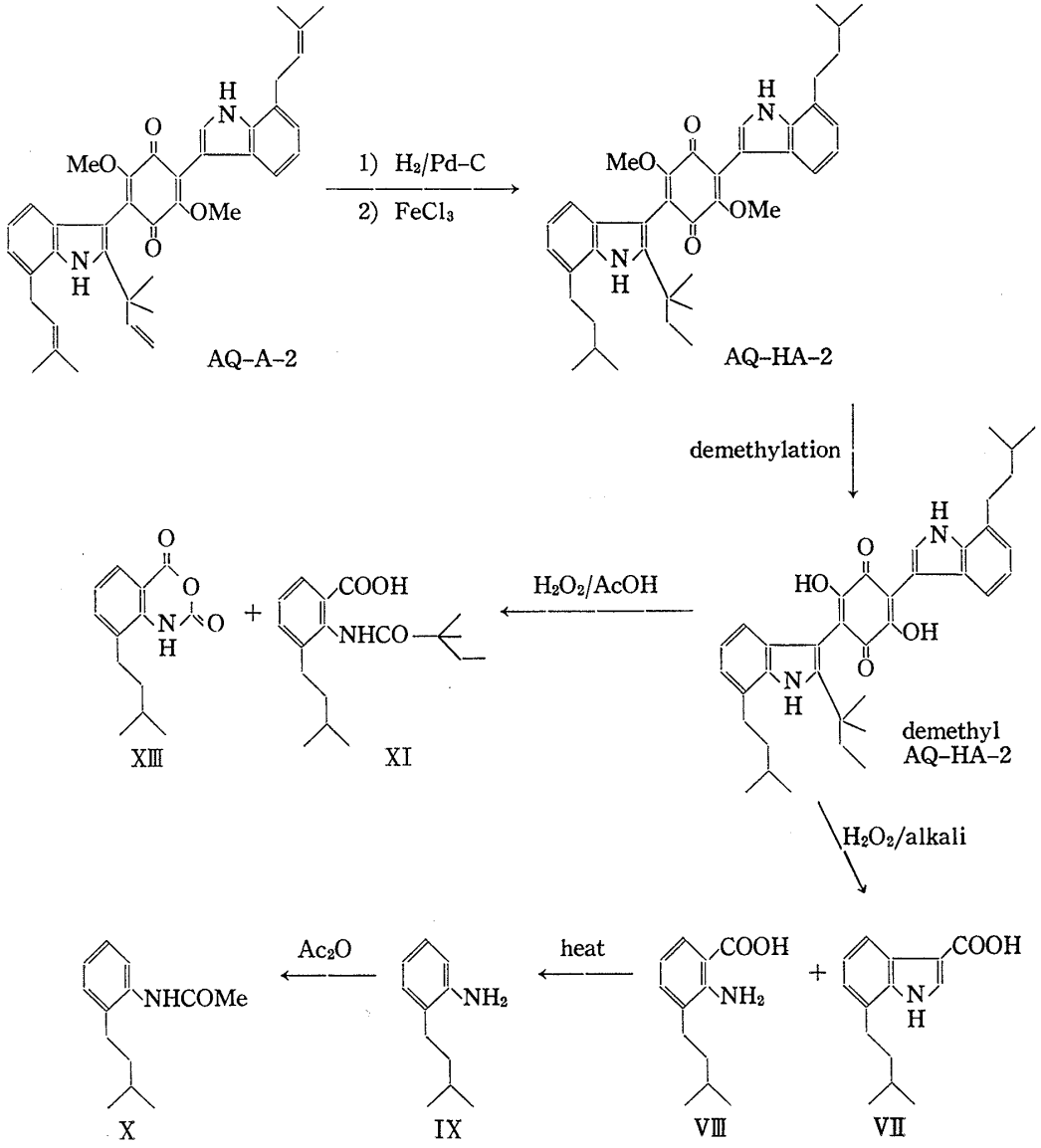


Fig. 5

Asterriquinone A-3 (AQ-A-3), obtained as a deep purple powder from benzene, had two 1,1-dimethylallyl groups. The substitution positions were proposed to be the N- and 2-atoms of the indole ring on the basis of the PMR spectrum (CH_3, δ 1.75 and 1.37, respectively). AQ-A-3 was converted to the corresponding quinone with saturated side chains (AQ-HA-3), and demethylated. The demethyl AQ-HA-3, mp 268—274° (dec.) was oxidized with hydrogen peroxide in an alkaline medium to afford N-*tert*-pentylindole-3-carboxylic acid (XII). When the demethylation of AQ-HA-3 was performed with hydrochloric acid, the N-*tert*-pentyl group was partially eliminated to give demethyl AQ-HC-1. From these results, the chemical

structure of AQ–A-3 was determined to be as shown in Fig. 4.

Asterriquinone A-4 (AQ–A-4), obtained as reddish-purple prisms from benzene, had two C-substituted 1,1-dimethylallyl groups (CH_3, δ 1.45 and 1.54) and one 3,3-dimethylallyl group. The side chain of AQ–A-4 was catalytically hydrogenated, followed by re-oxidation to the quinone (AQ–HA-4), mp 267—270°. AQ–HA-4 was demethylated, and oxidized with hydrogen peroxide in acetic acid. One of the products of the reaction was identical with V, and the other was identical with XI. From these results, the chemical structure of AQ–A-4 was determined to be as shown in Fig. 4.

All the asterriquinones and their derivatives were tested for antitumor activity, and the results will be reported in a separate paper.

Experimental[8]

Culture Conditions——*Aspergillus terreus* var. *africanus* IFO 8835 was cultivated stationarily in 500 ml Roux flasks containing 200 ml of the culture medium: glucose, 20 g; malt extract (Difico), 20 g; polypeptone (Daigoeiyo), 6 g; tap water, 1 l. After cultivation for 14 days at 27°, the mycelia were harvested by filtration and dried with warm air (90 g from 6 l of culture medium).

Extraction of Metabolites from Mycelia——The dried mycelia (90 g) were powdered and extracted with petr. ether and then ether in Soxhlet apparatus for 15 hr each. There were so many kinds of homologous pigments in these extracts that the isolation of each pigment was rather troublesome. Moreover, a part of the pigments was present in quinol form which complicated the separation.

The pigments in the petr. ether extract mainly consisted of the AQ-A group, and they were fractionated into four pigments, A-1, A-2, A-3, and A-4, as described below.

The ether extract was washed with 10% Na_2CO_3 to remove acidic compounds such as the butyrolactone derivative (II). When the extract was stored overnight, citreoviridin (III) separated out from the ether solution. III was crystallized from MeOH as bright yellow prisms, mp 105—107° (ref.[4] mp 107—111°). *Anal.* Calcd for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.28; H, 7.56. MS *m/e*: 402 (M^+), 340, 302, 259, 139. UV λ_{max}^{EtOH} nm: 288, 296, 390, 400 (sh). $[\alpha]_D^{16°}$ −105° (c=1.0, MeOH).

After removal of III, the ether layer was evaporated to dryness. The oily residue was refluxed with *n*-hexane. By this procedure, the soluble asterriquinone A group was separated from other pigments.

Fractionation of the Asterriquinone A Group——The fractions containing AQ-A group were combined, evaporated to dryness, and treated with MeOH. Insoluble oily compounds were discarded, and the solution was concentrated. Separated ergosterol was filtered off, and the mother liquor was fractionated by silica gel[9] chromatography with benzene. After elution of an oil, stearic acid, and a colorless compound, mp 159° (see Part VI of this series), AQ-A-1 was eluted. AQ-A-1 was purified by crystallization from MeOH as purple needles, mp 167—168° (yield,[10] 24 mg). *Anal.* Calcd for $C_{34}H_{34}N_2O_4$: C, 76.38; H, 6.41, N, 5.24. Found: C, 76.63; H, 6.47; N, 5.08.

The next fraction consisted mainly of AQ-A-2, which was crystallized from isopropyl alcohol as purple needles, mp 189—192° (yield, 28 mg). *Anal.* Calcd for $C_{39}H_{42}N_2O_4$: C, 77.71; H, 7.02; N, 4.65. Found: C, 77.76; H, 6.98; N, 4.53.

The last fraction was a mixture of A-3 and A-4. The mixture was fractionated by rechromatography on alumina with benzene–AcOEt (19: 1). In this case, A-4 was eluted before A-3. Sometimes, these pigments were separated by fractional crystallization from MeOH, in which AQ-A-4 was less soluble.

AQ-A-3, deep-purple powder from *n*-hexane, mp 112—114° (yield, 25 mg). *Anal.* Calcd for $C_{34}H_{34}N_2O_4$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.13; H, 6.49; N, 5.31.

AQ-A-4, reddish-purple prisms from MeOH, mp 252—253° (yield, 21 mg). *Anal.* Calcd for $C_{39}H_{42}N_2O_4$: C, 77.71; H, 7.02; N, 4.65. Found: C, 77.55; H, 7.03; N, 4.79.

Fractionation of the B, C, and D Groups——The ether extract from mycelia (5 g from 6 l of culture medium) was treated with *n*-hexane, and the insoluble part was roughly fractionated on a silica gel column (3.5×60 cm) with a mixture of benzene–AcOEt. B-2, a mixture of B-1, B-3, and B-4, C-1, a mixture of C-1 and C-2, and D were eluted in this order.

AQ-B-2 was crystallized from benzene as deep-purple prisms, mp 199—200° (yield, 380 mg). *Anal.* Calcd for $C_{34}H_{34}N_2O_4$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.13; H, 6.44; N, 4.97.

The mixture of B-1, B-3, and B-4 was combined with the mother liquor of B-2 and re-chromatographed on an alumina column (3×30 cm) with benzene–AcOEt. In this case, pigments were eluted in the order of B-1, B-2, and a mixture of B-3 and B-4.

AQ-B-1 was crystallized from benzene as deep-purple prisms, mp 208—209° (yield, 290 mg). *Anal.* Calcd for $C_{34}H_{34}N_2O_4$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.00; H, 6.36; N, 5.09.

The mixture of B-3 and B-4 was separated by fractional crystallization from MeOH, in which B-4 was less soluble. AQ-B-3, reddish-purple powder from cyclohexane, mp 148—150° (yield, 120 mg). *Anal.* Calcd for $C_{29}H_{26}N_2O_4$: C, 74.66; H, 5.62; N, 6.01. Found: C, 74.55; H, 5.80; N, 6.07.

AQ-B-4, reddish-purple plates from MeOH, mp above 300° (yield, 72 mg). *Anal.* Calcd for $C_{34}H_{34}N_2O_4$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.21; H, 6.18; N, 5.24.

C-1 was easily crystallized from benzene, so the mixed fraction of C-1 and C-2 was treated with benzene, and the mother liquor enriched with C-2 was chromatographed on alumina with benzene–AcOEt (9: 1). C-1 was isolated from the first purple fraction, and C-2 was obtained from the second reddish-purple fraction.

AQ-C-1, purple prisms from benzene, mp 213—214° (yield, 1.4 g). *Anal.* Calcd for $C_{29}H_{26}N_2O_4$: C, 74.66; H, 5.62; N, 6.01. Found: C, 74.39; H, 5.70; N, 5.68.

AQ-C-2, crystallized from MeOH as purple needles, mp 202—203° (yield, 150 mg). *Anal.* Calcd for $C_{29}H_{26}N_2O_4$: C, 74.66; H, 5.62; N, 6.01. Found: C, 74.57; H, 5.46; N, 5.70.

AQ-D was the most insoluble in organic solvents, and sometimes separated out during elution. The crude AQ-D was washed with ether and crystallized from acetone as a deep-purple powder, mp above 300° (yield, 110 mg). *Anal.* Calcd for $C_{24}H_{18}N_2O_4$: C, 72.35; H, 4.55; N, 7.03. Found: C, 72.28; H, 4.52; N, 7.29.

Demethylation of Asterriquinone A-1 to Asterriquinone (I)——AQ-A-1 (250 mg) was dissolved in acetone (15 ml) and 10% HCl (7 ml) was added. After being boiled for 8 hr, the reaction mixture was poured into ice-water. The precipitate was dissolved in ether, and extracted with 10% $NaHCO_3$ and then 10% Na_2CO_3. The Na_2CO_3 extract was acidified, extracted with ether, and the ether extract was chromatographed on oxalic acid-treated silica gel[11] with benzene to obtain I, mp 218—220° (dec.) (yield, 20 mg).

The $NaHCO_3$ fraction gave three compounds which will be described in Part V of this series.

Oxidation of Demethyl AQ-C-1 with Dimethyl Sulfoxide in Ac_2O——Demethyl AQ-C-1 (100 mg) was dissolved in DMSO (1 ml) and Ac_2O (1.5 ml). After being heated at 120° for 4 min, the reaction mixture was poured into H_2O, and the resulting precipitate was purified by silica gel chromatography. Upon elution with benzene, an orange-red compound was obtained. It was crystallized from CH_2Cl_2 as orange-red needles (VI) (32 mg), mp above 300°. *Anal.* Calcd for $C_{27}H_{20}N_2O_4$: C, 74.30; H, 4.62; N, 6.42. Found: C, 74.02; H, 4.51; N, 6.31. MS *m/e* 436 (M^+). UV λ_{max}^{EtOH} nm (log ε): 274, 283 (4.32), 290, 480 (4.22).

Preparation of Asterriquinones with Saturated Side Chains——Hydrogenation of AQ-B-1 is described as an example. AQ-B-1 (500 mg) was dissolved in AcOEt (150 ml), and catalytically hydrogenated with 5% Pd-C (500 mg). Three moles of H_2 was absorbed, and the solution became colorless. The catalyst was filtered off and the solvent was evaporated off under reduced pressure. The residue was dissolved in MeOH (20 ml), and the solution was shaken with aq. 5% $FeCl_3$ for 10 min. The purple solution was poured into H_2O, and the resulting precipitate was crystallized from MeOH as dark-purple prisms, mp 255—257° (AQ-HB-1). *Anal.* Calcd for $C_{34}H_{38}N_2O_4$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.57; H, 7.15; N, 5.22.

The hydrogenated quinones were demethylated with acid or alkali (see Part V of this series) to the corresponding demethyl compounds, which were then degraded.

Oxidative Degradation of Demethyl Hydrogenated Asterriquinones in Alkaline Medium——The reaction of AQ-HB-1 is described as an example. Demethyl AQ-HB-1 (100 mg) was dissolved in 0.1 N NaOH (20 ml) and oxidized with 30% H_2O_2 (10 ml). After standing at room temperature for 3 hr, the solution was acidified and extracted with AcOEt. The AcOEt extract was separated by preparative TLC (silica gel, *n*-hexane–AcOEt, 1: 1) into three components (*Rf*, 0.74, 0.63, and 0.43).

From the highest band (*Rf*, 0.74), 3-isopentylanthranilic acid (VIII) was isolated as colorless needles from petr. benzin, mp 123—124° (yield, 5 mg). *Anal.* Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.47; H, 8.36; N, 6.76. MS *m/e*: 207 (M^+). PMR (CCl_4) δ: 1.0 (6H, d, J=8.0 Hz), 1.16—1.87 (3H, m), 2.47 (2H, t, J=8.0 Hz), 6.43 (1H, dd, J=7.2, 8.0 Hz), 7.00 (1H, dd, J=7.2, 1.6 Hz), 7.70 (1H, dd, J=8.0, 1.6 Hz). IR ν_{max}^{KBr} cm^{-1}: 3480, 3350, 3050—2500, 1670. The same compound was also obtained from demethyl AQ-HA-2 by the same reaction.

Compound VIII (56 mg) was decarboxylated by heating at 210° for 1.5 hr. The reaction mixture was dissolved in ether and washed with 1% NaOH. The ether was evaporated off and the brownish residue was acetylated with Ac_2O (5 ml) at 75°. The acetate was extracted with ether, and the ether solution was washed with 10% $NaHCO_3$, then evaporated to dryness, and the residue was purified by preparative TLC (silica gel; benzene–AcOEt, 1: 3). A colorless compound, mp 98—99° (yield, 35 mg) was obtained as needles from *n*-hexane. *Anal.* Calcd for $C_{13}H_{10}NO$: C, 76.05; H, 9.33; N, 6.82. Found: C, 76.10; H, 9.44; N, 7.03. This compound was identified as *o*-isopentylacetanilide (X) by IR and PMR spectral comparison and mixed melting point determination.

The second product from the band at *Rf* 0.63 was crystallized from petr. ether as colorless needles, mp 137—138°. *Anal.* Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.26; H, 7.51; N, 5.85. MS *m/e*: 235 (M^+). This compound was identical with N-(2,2-dimethylbutyroyl)anthranilic acid (V). The same compound was also obtained from demethyl AQ-HA-3 or HB-4 by the same reaction. The acyl compound (V) (30 mg) was hydrolyzed with boiling 10% HCl (5 ml) under an N_2 atmosphere for 4 hr. The reaction mixture was adjusted to pH 6, and extracted with AcOEt. The AcOEt extract was crystallized from *n*-hexane as a colorless powder, mp 142—144° (yield, 8 mg), which was identified as anthranilic acid by IR spectral comparison and mixed melting point determination.

The third compound from the band at *Rf* 0.43 was crystallized from benzene as colorless prisms, mp 195—196° (yield, 5 mg). It was positive to the Ehrlich reaction. *Anal.* Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.35; H, 7.15; N, 5.79. It was identified as 7-isopentylindole-3-carboxylic

acid (VII). This compound was also obtained from demethyl AQ-HA-2 or HC-2 by the same methods.

Indole-3-carboxylic acid (IV), mp 212—214° was also obtained from demethyl AQ-HB-2, HB-3, HC-1, HC-2 and D under the same alkaline conditions. IV was isolated by preparative TLC (silica gel, *n*-hexane–AcOEt, 1: 1) from the band at *Rf* 0.25.

When demethyl AQ-HB-3 was oxidized with H_2O_2 under alkaline conditions, colorless prisms, mp 152—153°, were obtained from the acidic fraction. This compound was identified as N-*tert*-pentylindole-3-carboxylic acid (XII), which had been obtained from asterriquinone (I) and demethyl AQ-HA-3. *Anal.* Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.93; H, 7.45; N, 5.84. MS *m/e*: 231 (M^+).

Oxidative Degradation of Demethyl Hydrogenated Asterriquinones in Acetic Acid Medium——Demethyl AQ-HB-2 (100 mg) was dissolved in glacial acetic acid (5 ml), and 30% H_2O_2 (1.6 ml) was added. After standing at room temperature for 24 hr, the orange-red solution was poured into H_2O, and the mixture was extracted with AcOEt. The solvent was evaporated off *in vacuo*, and the residue was extracted with petr. benzin. The soluble part was separated by preparative TLC (acid-washed silica gel; *n*-hexane–AcOEt, 1: 1). The band at *Rf* 0.59 was extracted and crystallized from petr. benzin as colorless needles, mp 116—117°. *Anal.* Calcd for $C_{18}H_{27}NO_3$: C, 70.79; H, 8.91; N, 4.59. Found: C, 70.65; H, 8.91; N, 4.78. IR ν_{max}^{KBr} cm^{-1}: 3300, 1700, 1653. This compound was identified as 3-isopentyl-N-(2,2-dimethylbutyroyl)anthranilic acid (XI). XI (35 mg) was treated with boiling 6 N HCl (10 ml) for 8 hr under an N_2 atmosphere. The reaction mixture was adjusted to pH 6.0, and extracted with AcOEt. The product was identified as 3-isopentylanthranilic acid (VIII), which was also obtained from demethyl AQ-HB-1 by H_2O_2 oxidation in an alkaline medium.

Demethyl AQ-HA-2 was oxidized with H_2O_2 in AcOH by the method described for demethyl AQ-HB-2. The product was purified by preparative TLC (acid-washed silica gel; *n*-hexane–AcOEt, 1: 1). VIII was obtained from the fraction at *Rf* 0.61, and colorless prisms, mp 175—177.5°, were isolated from the band at *Rf* 0.77 by crystallization from benzene. The latter product was identified as 3-isopentylisatonic acid anhydride on the basis of the physical properties and elementary analysis. *Anal.* Calcd for $C_{13}H_{15}NO_3$: C, 66.93; H, 6.48; N, 6.01. Found: C, 66.94; H, 6.43; N, 6.04. MS *m/e*: 233 (M^+), 187, 174, 161, 146. UV λ_{max}^{EtOH} nm (log ε): 240, 322 (4.57). IR ν_{max}^{KBr} cm^{-1}: 3160, 1795, 1730.

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References and Notes

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8) All melting points are uncorrected.
9) Silica gel (Kanto Chemical, for chromatography) was used in all experiments.
10) The yields of all asterriquinones and other metabolites showed by weight were those obtained from 6 l of culture medium.
11) Silica gel (Kanto Kemical, for chromatography) was suspended in 0.1 M oxalic acid overnight, filtered, washed with H_2O and dried in an oven at 100°.