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# Metabolic Products of *Aspergillus terreus*. V.[1] Demethylation of Asterriquinones

Kunizo Arai, Sakae Shimizu, Yasuhisa Taguchi, and Yuzuru Yamamoto*

*Faculty of Pharmaceutical Sciences, Kanazawa University, Takaramachi 13-1, Kanazawa 920, Japan*

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Demethylation of asterriquinones (AQ, bisindolyl-dimethoxyl benzoquinones) was investigated in connection with the determination of their structures and studies of their antitumor activity. Usually, the demethylation proceeded smoothly with KOH, but several types of side reactions were observed with acidic reagents.

**Keywords**——demethylation; asterriquinones; *Aspergillus terreus*; IFO 8835; benzoquinone; indolyl; dimethylallyl

In the previous paper,[1] we reported the isolation and chemical structures of 11 kinds of asterriquinones (AQ–A-1 through AQ–D) produced by *Aspergillus terreus* var. *africanus* IFO 8835.

The determination of the chemical structures of these pigments was usually performed by cleavage of the quinone ring with hydrogen peroxide. Before oxidation, dimethylallyl groups of the pigments were usually hydrogenated, and methoxyl groups were demethylated, because the presence of methoxyl groups on the benzoquinone ring resulted in low yields of degradation products, and the presence of double bonds in the dimethylallyl group caused various types of side reactions as described below.

Demethyl compounds were also necessary for the investigation of antitumor activity, because hydroxyl benzoquinone derivatives were found to be more effective than methoxyl derivatives.[2] Thus, we extensively studied the demethylation of asterriquinones and their hydrogenated derivatives.

Demethylation was investigated with several reagents, such as hydrochloric, hydrobromic, and hydroiodic acids, boron tribromide, aluminum tribromide, and potassium hydroxide under various conditions. The reaction with boron tribromide gave considerable amounts of partially demethylated compounds, but with other acidic reagents, there were many side reactions, especially in the case of pigments having unsaturated side chains. On the other hand, de-

Table I. Demethylation of Asterriquinones (AQ) with KOH

| Starting AQ | Reaction Time[a] (hr) | Yield (%) | Demethyl AQ Formula[b] | mp (C°) |
|---|---|---|---|---|
| AQ–A-1 | 1.5 | 72 | $C_{32}H_{30}N_2O_4$ | 218—220 |
| AQ–A-2 | 1 | 56 | $C_{37}H_{38}N_2O_4$ | 169—171 |
| AQ–A-3 | 1 | 60 | $C_{32}H_{30}N_2O_4$ | 260—262 |
| AQ–A-4 | 2.5 | 85 | $C_{37}H_{38}N_2O_4$ | 111—115 |
| AQ–B-1 | 1.1 | 63 | $C_{32}H_{30}N_2O_4$ | 199—201 |
| AQ–B-2 | 1 | 76 | $C_{32}H_{30}N_2O_4$ | 192—195 |
| AQ–B-3 | 1 | 14 | $C_{27}H_{22}N_2O_4$ | 222—224 |
| AQ–B-4 | 2.5 | 83 | $C_{32}H_{30}N_2O_4$ | 245—246 |
| AQ–C-1 | 1.1 | 82 | $C_{27}H_{22}N_2O_4$ | 244—246 |
| AQ–C-2 | 1 | 58 | $C_{27}H_{22}N_2O_4$ | >300 |
| AQ–D | 1 | 59 | $C_{22}H_{14}N_2O_4$ | >300 |

*a*) Refluxing time with 1 N KOH and EtOH (1: 2, v/v).
*b*) Elementary analyses and MS were all consistent with the expected values.

methylation with potassium hydroxide in boiling ethanol gave good yields. The results of demethylation of asterriquinones with potassium hydroxide are summarized in Table I.

Asterriquinone B-3 (AQ–B-3) gave an exceptionally low yield (14%) in demethylation with potassium hydroxide, despite of the use of various conditions. Therefore, the required amount for antitumor tests was prepared by treatment with hydrochloric acid in acetic acid (yield, 23%).

Generally, the 1,1-dimethylallyl group at the 2-position of an indole ring was fairly stable even in boiling acidic reagents.[3] On the other hand, the N-1,1-dimethylallyl group readily underwent side reactions such as elimination and rearrangement.

The 3,3-dimethylallyl group at position 7 also underwent side reactions such as cyclization with the N atom of an indole ring or addition of water to give a side chain having a *tert*-hydroxyl group. This tendency was more marked in the reaction with hydrobromic acid than with hydrochloric acid.

The side reactions observed during demethylation of asterriquinones were classified as follows: i) partial demethylation, ii) elimination of dimethylallyl groups, iii) rearrangement of dimethylallyl group, iv) hydration of 3,3-dimethylallyl group, and v) cyclization of dimethylallyl group.

### i) Partial Demethylation

Upon treatment with boron tribromide in dichloromethane, asterriquinones gave mono-demethyl derivatives. For example, AQ–C-1 gave mono-demethyl AQ–C-1, mp 154° (yield, 32%), and the di-demethyl compound, mp 244—246° (yield, 18%). AQ–B-1 with its saturated side chain (AQ–HB-1) gave the mono-demethyl compound in 46% yield by the same method. Other pigments such as AQ–B-3 or AQ–D gave smaller amounts of the mono-demethylated compounds. Thus, the bulky 1,1-dimethylallyl group at the 2-position of the indole ring seemed to prevent the access of the reagent to methoxyl groups on the benzoquinone ring.

### ii) Elimination of Dimethylallyl Groups

Fujino[4] reported that N-*tert*-pentyltryptophan lost the substituent group to give tryptophan, but an N-1,1-dimethylallyl group was cyclized by treatment with hydrochloric acid in acetic acid at 100° as shown in Fig. 1.

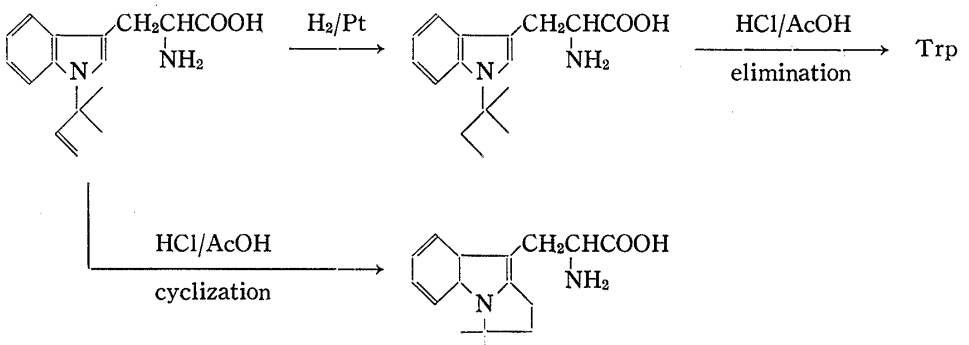


Fig. 1

The same elimination reactions also occurred in asterriquinones with N-*tert*-pentyl groups. Thus, AQ–HA-3 afforded demethyl AQ–HC-1 in high yield (57%), and AQ–HB-3 gave demethyl AQ–D (yield, 3%) on treatment with boiling hydrochloric acid in acetic acid.

The elimination of N-1,1-dimethylallyl groups was also observed upon refluxing the compounds with hydrochloric acid in acetone. For example, AQ–A-1 lost one of the N-1,1-dimethylallyl groups to give demethyl AQ–B-3 (yield, 22%). AQ–B-3 was deprenylated to demethyl AQ–D (yield, 24%). These reactions were very useful for determination of the chemical relations among these pigments, which are summarized in Fig. 2.

### iii) Rearrangement of Dimethylallyl Groups

On refluxing with hydrochloric acid in acetone, AQ-B-3 gave compound I, which was isolated by silica gel chromatography as a purple powder, mp 212—214° (yield, 11%). Compound I had the same molecular formula as demethyl AQ-B-3, $C_{27}H_{22}N_2O_4$, and the presence of a 3,3-dimethylallyl group at position 2 of the indole ring instead of the N-1,1-dimethylallyl

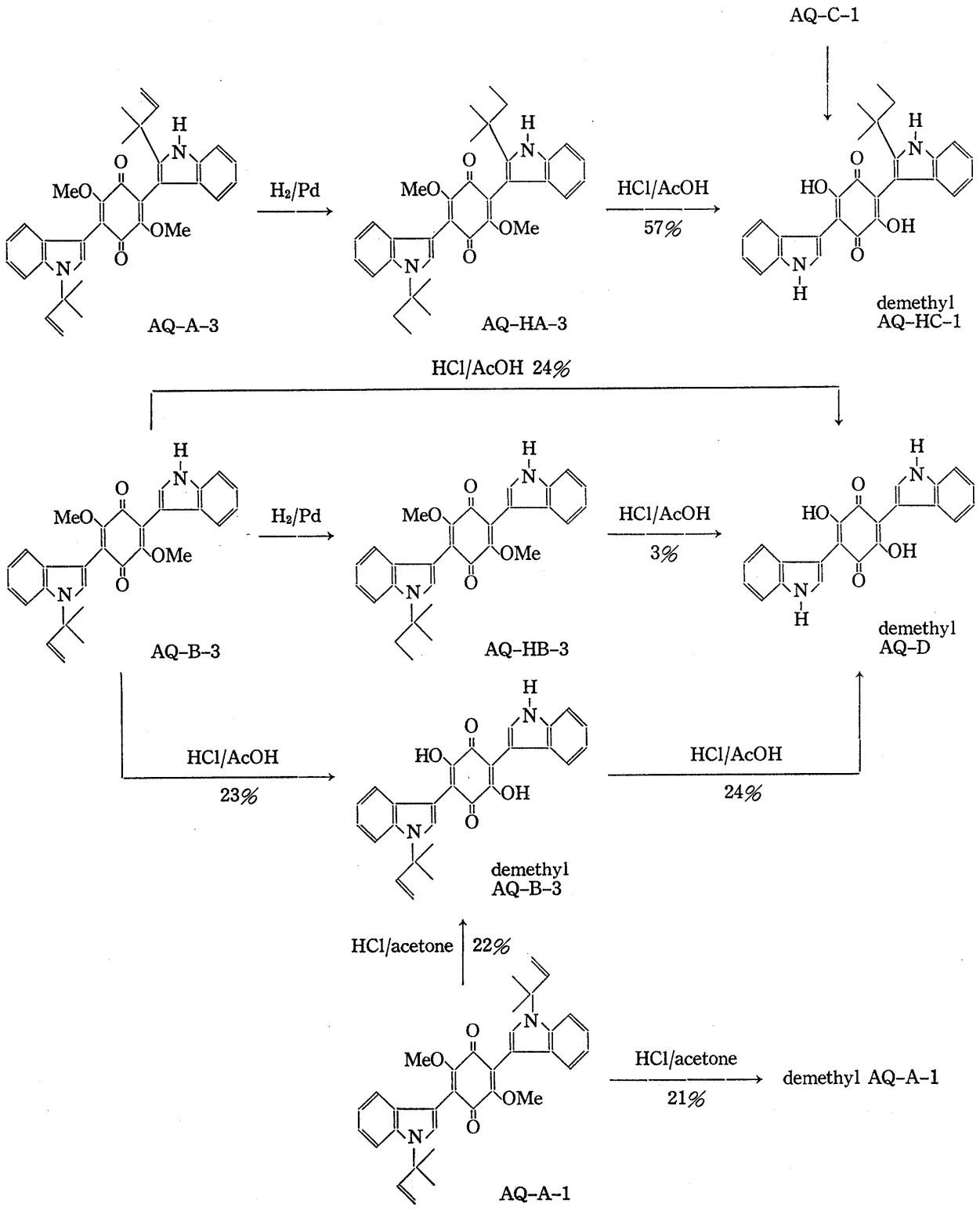


Fig. 2

group was suggested by the proton nuclear magnetic resonance (PMR) and infrared (IR) spectra. The rearrangement was confirmed by the following determination of the structure of compound I. This compound was converted to the pentyl derivative (II), mp 219—221° (dec.), $C_{27}H_{24}N_2O_4$, by hydrogenation followed by oxidation with ferric chloride . Compound II was oxidized with hydrogen peroxide in acetic acid to provide III, mp 82—83°, $C_{13}H_{17}NO_3$. Compound III was identified as N-(4,4-dimethylbutyroyl)anthranilic acid, which was hydrolyzed to anthranilic acid, mp 144°. When compound I was oxidized with hydrogen peroxide in an alkaline medium, colorless needles (IV), mp 173—174°, $C_{14}H_{15}NO_2$, were obtained. This compound was identical with 2-(3,3-dimethylallyl)indole-3-carboxylic acid as judged by PMR, UV, and IR spectral comparisons.

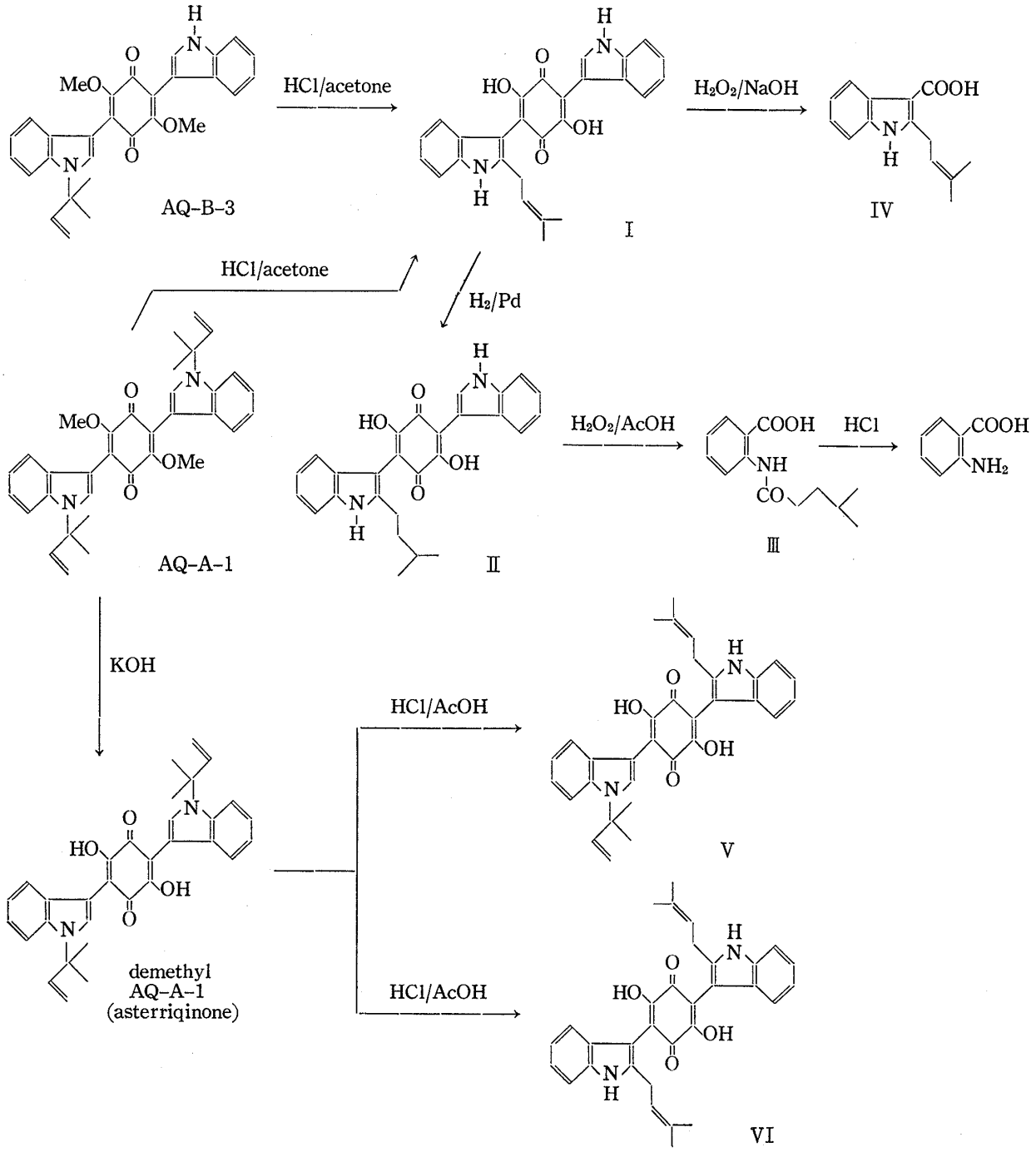


Fig. 3

These reactions are summarized in Fig. 3.

AQ–A-1 also gave compound I (yield, 5%) on refluxing with hydrochloric acid in acetone. In this case, two types of reactions of N-3,3-dimethylallyl groups occurred. One was elimination, and the other was rearrangement. This type of rearrangement was more significant in the reaction of demethyl AQ–A-1 (asterriquinone) with hydrochloric acid in acetic acid medium. The reaction proceeded very speedily at room temperature, and the mono-rearranged compound (V), mp 164—165° was obtained in 36% yield, while the di-rearranged compound (VI), mp 196—199° was isolated in 30% yield. The starting material was recovered (9.7%) with a trace amount of demethyl AQ–B-3 and demethyl AQ–D. These reactions are also shown in Fig. 3.

Casnati and Inada[5] reported that a 3,3-dimethylallyl group on the N atom of 3-methylindole was rearranged with inversion to yield 2-(1,1-dimethylallyl)-3-methylindole by treatment with an acidic catalyst ($CF_3COOH$ or $AlCl_3$). However, the rearrangement of an N-1,1-dimethylallyl group has not yet been reported. Thus, the reactions observed in AQ–B-3, A-1, and asterriquinone are new rearrangements.

### iv) Hydration of the 3,3-Dimethylallyl Group

Addition of a water molecule to a 3,3-dimethylallyl group at the 7 position was also observed. For instance, AQ–B-1, AQ–B-2, and AQ–C-2 gave the corresponding *tert*-alcohols,

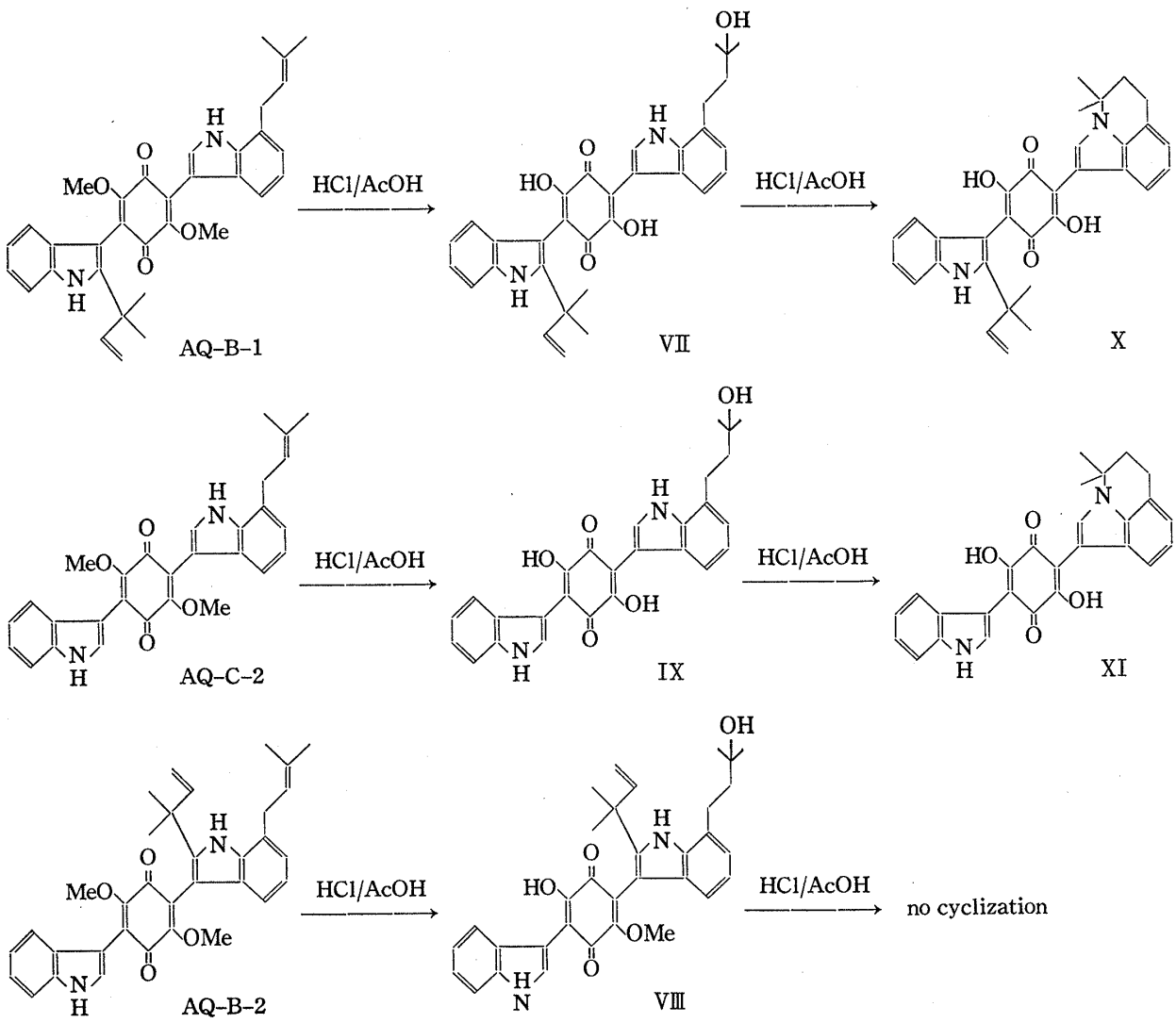


Fig. 4

VII, VIII, and IX, respectively, upon treatment with boiling hydrochloric acid or hydrobromic acid in acetic acid. The yields were fairly high (42% in the case of VIII), but the 1,1-dimethylallyl group did not undergo this reaction. A similar reaction was reported in terpenoids and flavonoids (*cf.* glycyrin[6]). The structures of these *tert*-alcohols were easily determined by PMR, IR, and mass spectroscopy. These reactions are summarized in Fig. 4.

### v) Cyclization

Cyclization between the 3,3-dimethylallyl group at position 7 and the N atom of the indole ring was also observed. AQ–B-1 and AQ–C-2 gave the cyclic compounds X (yield, 10%) and XI (yield, 14%), respectively, on being heated with hydrochloric acid in acetic acid. However, AQ–B-2 gave no cyclized compound in spite of the presence of a 3,3-dimethylallyl group at position 7. The steric hindrance due to the bulky 1,1-dimethylallyl group at position 2 of the same indole ring seemed to prevent the cyclization.

The cyclization between *ortho* hydroxyl and 3,3-dimethylallyl group is well known (*cf.* a butyrolactone derivative isolated from this fungus,[7] and aspulvinones,[8]) and the cyclization between position 2 and an N-1,1-dimethylallyl group in an indole ring was also reported.[4] However, this is the first report of the cyclization between a 7-(3,3-dimethylallyl) group and the N atom of an indole ring.

The hydrate of AQ–B-1 was changed to the corresponding cyclic compound by treatment with hydrochloric acid in acetic acid, which suggested that the cyclization proceeded *via* the *tert*-alcohol (see Fig. 4).

The compounds obtained in this paper were all tested for antitumor activity. The results will be reported in a separate paper.

## Experimental[9]

**Demethylation of AQ-B-2 with KOH**——AQ-B-2 (100 mg) was dissolved in 1 N KOH (10 ml) and EtOH (20 ml), and the solution was refluxed for 1 hr. The solvent was evaporated off *in vacuo*, and the residue was acidified with HCl after dilution with $H_2O$. The precipitate was collected, dissolved in ether, and extracted with 10% $Na_2CO_3$. The alkaline solution was acidified, and the resulting precipitate was chromatographed on silica gel[10] (solvent: benzene–AcOEt, 19: 1). The purple eluate was evaporated to dryness and the residue was crystallized from benzene as a dark-purple powder, mp 192—195° (yield, 72 mg, 76%). *Anal.* Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.57; H, 5.83; N, 5.35. MS *m/e*: 506 ($M^+$).

**Demethylation of AQ-C-1 with HCl in AcOH**——AQ-C-1 (100 mg) was dissolved in 10% HCl (8 ml) and AcOH (15 ml), and the solution was refluxed for 30 min. The reaction mixture was neutralized with NaOH, and extracted with ether. The ether solution was extracted with 10% $NaHCO_3$ and then with 10% $Na_2CO_3$. Unchanged AQ-C-1 was recovered from the ether solution. The $NaHCO_3$ fraction was purified by silica gel chromatography (benzene–AcOEt, 9: 1). Demethyl AQ-C-1 was obtained as purple needles by crystallization from cyclohexane–isopropyl ether, mp 244—246° (yield, 33 mg, 35%). *Anal.* Calcd for $C_{27}H_{22}N_2O_4$: C, 73.96; H, 5.06; N, 6.39. Found: C, 74.10; H, 4.86; N, 6.21.

From the $Na_2CO_3$ fraction, mono-demethyl AQ-C-1, mp 153—154° was obtained (yield, 3%). This compound was also obtained by treatment with $BBr_3$.

Demethylation of AQ-C-1 also proceeded with HBr under the same conditions, and the yield of the demethyl compound was somewhat higher (47%).

**Demethylation of Hydro-AQ-A-2 (AQ-HA-2) with HBr**——AQ-HA-2 (100 mg) was dissolved in a mixture of AcOH (14 ml), acetone (2 ml), and 10% HBr (6 ml). After refluxing for 50 min, the reaction mixture was poured into $H_2O$. The resulting precipitate was dissolved in ether, and extracted with 0.1 N NaOH. The alkaline solution was acidified and the resulting precipitate was chromatographed on silica gel (benzene–AcOEt, 19: 1). The purple eluate was concentrated and crystallized from benzene. Demethyl AQ-HA-2 was obtained as purple prisms, mp 190.5—192° (yield, 60 mg, 63%). *Anal.* Calcd for $C_{37}H_{44}N_2O_4$: C, 76.52; H, 7.64; N, 4.82. Found: C, 76.65; H, 7.69; N, 5.00.

**Demethylation of AQ-C-1 with $BBr_3$ (Partial Demethylation)**——$BBr_3$ (5.3 mol equivalent) in $CH_2Cl_2$ (20 ml) was added slowly at −70° to −80° to a solution of AQ-C-1 (300 mg) in dry $CH_2Cl_2$ (60 ml). The initial purple color changed to dark green when the solution was allowed to stand for 1 hr at this temperature. After standing for a further 1 hr at room temperature, the reaction mixture was poured into $H_2O$, and extracted with ether. The ether solution was extracted with 10% $NaHCO_3$ and then with 10% $Na_2CO_3$. These alkaline solutions were acidified and the resulting precipitate was purified by silica gel chromatography.

From the $NaHCO_3$ fraction, demethyl AQ-C-1, mp 244—246° was obtained (yield, 50 mg, 18%) by elution with benzene–AcOEt (19: 1). The $Na_2CO_3$ fraction was eluted with benzene–AcOEt (9: 1), and purple prisms, mp 153—154° (mono-demethyl AQ-C-1), were obtained by crystallization from benzene (yield, 93 mg, 32%). *Anal.* Calcd for $C_{28}H_{24}N_2O_4$: C, 74.32; H, 5.35; N, 6.19. Found: C, 74.34; H, 5.52; N, 5.96.

The amount of $BBr_3$ was varied from 4.0 to 7.0 mol, but the yields of the two compounds were not much affected.

**Demethylation of Hydro-AQ-A-3 (AQ-HA-3) with HCl (Elimination of the N-1,1-Dimethylallyl Group)**——A solution of AQ-HA-3 (100 mg) in AcOH (7 ml) and 10% HCl (3 ml) was refluxed for 20 min. The reaction mixture was poured into $H_2O$, and the resulting precipitate was dissolved in ether, and extracted with 10% $Na_2CO_3$. The acidic compound obtained from the $Na_2CO_3$ extract was purified by silica gel chromatography (benzene–AcOEt, 10: 1). The first purple eluate was evaporated to dryness and the residue was crystallized from benzene as dark-green needles (demethyl AQ-HA-3), mp 268—274° (dec.) (yield, 23 mg, 24%). *Anal.* Calcd for $C_{32}H_{34}N_2O_4$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.41; H, 6.71; N, 5.27. MS *m/e*: 510 ($M^+$), 490, 440. UV $\lambda_{max}^{EtOH}$ nm: 285, 292, 378, 563.

The second purple eluate was crystallized from benzene as purple needles, mp 240—242° (yield, 47 mg, 57%). This compound was identified as demethyl AQ-HC-1 by IR and PMR spectral comparison and mixed melting point determination.

**Demethylation of AQ-A-1 with HCl in Acetone (Elimination and Rearrangement of Dimethylallyl Groups)**——AQ-A-1 (250 mg) was refluxed in 10% HCl (7 ml) and acetone (14 ml) for 8 hr. The reaction mixture was poured into $H_2O$, and the precipitate was dissolved in ether. The solution was extracted with 10% $NaHCO_3$ and then with 10% $Na_2CO_3$. The acidic compound obtained from the $NaHCO_3$ fraction was chromatographed on silica gel (benzene–AcOEt, 10: 1). The first reddish-purple eluate was crystallized from MeOH as purple needles, mp 222—224° (dec.) (yield, 18 mg, 22%). It was identified as demethyl AQ-B-3 from its IR spectrum. The second fraction was crystallized from benzene as a purple powder, mp 212—214° (dec.) (yield, 4 mg, 4.9%). It was identical with compound I which was also obtained from AQ-B-3.

The $Na_2CO_3$ extract was also chromatographed on silica gel with benzene. The purple eluate was crystallized from MeOH as deep-purple needles, mp 218—220° (dec.) (yield, 20 mg, 21%). This compound was identified as demethyl AQ-A-1 (asterriquinone).

**Rearrangement of the Dimethylallyl Group in Demethyl AQ-A-1 (Asterriquinone)**——Demethyl AQ-A-1 (asterriquinone) (300 mg) was dissolved in AcOH (60 ml) and conc. HCl (6 ml), and left to stand at room temperature for 5 min. The reaction mixture was poured into $H_2O$, and the resulting purple precipitate was purified by chromatography on a silica gel column. On elution with benzene, starting material was recovered (yield, 9.7%). The column was further eluted with benzene–AcOEt (19: 1). From the first fraction, a dark-purple compound (V), mp 164—165°, and from the second fraction, a dark-purple compound (VI), mp 196—199°, were isolated. On further elution with solvent mixtures of benzene–AcOEt having higher AcOEt contents (10—20%), small amounts of demethyl AQ-B-3 and AQ-D were isolated.

Compound V was crystallized from cyclohexane as a dark-purple amorphous powder. *Anal.* Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.73; H, 6.10; N, 5.29. This compound had one N-1,1-dimethylallyl group and one 2-(3,3-dimethylallyl) group on the basis of the PMR spectrum. PMR ($CDCl_3$) $\delta$: 1.73 (6H, s), 3.40 (2H, d, $J$=7.0 Hz), 5.32 (1H, t, $J$=7.0 Hz) (3,3-dimethylallyl group at C-2 of indole ring); 1.80 (6H, s), 5.16 (1H, d, $J$=18 Hz), 5.21 (1H, d, $J$=10 Hz), 6.16 (1H, dd, $J$=10, 18 Hz) (N-1,1-dimethyllallyl group); 6.94—7.71 (8H, m, aromatic H), 7.14 (1H, s, 2-CH of indole ring), 8.01 (3H, bs, 2 OH and NH).

Compound VI was crystallized from benzene as a dark-purple powder. *Anal.* Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.71; H, 5.94; N, 5.66. The symmetrical structure and the presence of two 3,3-dimethylallyl groups were suggested by the PMR spectrum. PMR ($CDCl_3$) $\delta$: 1.75 (6H, $2CH_3$), 1.79 (6H, $2CH_3$), 3.41 (4H, d, $J$=8.0 Hz, $2CH_2$), 5.33 (2H, t, $J$=8.0 Hz, 2CH) (two 3,3-dimethylallyl groups at C-2), 6.95—7.36 (8H, m, aromatic H), 7.89 (2H, bs, 2OH), 8.07 (2H, bs, 2NH).

**Demethylation of AQ-B-3 with HCl in Acetone (Elimination and Rearrangement of Dimethylallyl Group)**——AQ-B-3 (320 mg) was refluxed with 10% HCl (9 ml) and acetone (20 ml) for 5 hr. The reaction mixture was poured into $H_2O$. The resulting precipitate was dissolved in ether, and extracted with 10% $NaHCO_3$. The alkaline fraction was chromatographed on silica gel (benzene–AcOEt, 10: 1). The first eluate was crystallized from MeOH to provide deep-purple needles (demethyl AQ-B-3), mp 222—224° (dec.) (yield, 41 mg, 14%). *Anal.* Calcd for $C_{27}H_{22}N_2O_4$: C, 73.96; H, 5.06; N, 6.39. Found: C, 74.01; H, 4.97; N, 6.60.

The second fraction was crystallized from benzene as a deep-purple powder (I), mp 212—214° (dec.) (yield, 33 mg, 11%). *Anal.* Calcd for $C_{27}H_{22}N_2O_4$: C, 73.96; H, 5.06; N, 6.39. Found: C, 73.83; H, 5.06; N, 6.34. MS *m/e*: 438 ($M^+$), 371. PMR (acetone-$d_6$) $\delta$: 1.67 (6H, d, $J$=1.2 Hz), 3.45 (2H, d, $J$=7.0 Hz), 5.34 (1H, t, $J$=7.0 Hz) (3,3-dimethylallyl group at C-2), 6.83—7.60 (9H, m, aromatic H), 9.25 (2H, bs, 2 OH), 9.98 (1H, bs, NH), 10.40 (1H, bs, NH).

The third fraction was concentrated and crystallized from acetone as deep-purple needles, mp above 300° (yield, 60 mg, 24%). It was identified as demethyl AQ-D from its IR and PMR spectra.

**Determination of the Structure of I**——The chemical structure of I was determined by the following degradation. Compound I (150 mg) was catalytically hydrogenated with Pd–C in AcOEt (100 ml). After 2 mol

of $H_2$ had been absorbed, the catalyst was filtered off, and the solvent was evaporated off. During the operation, the purple color was regenerated by air oxidation, and the dihydro derivative (II), mp 219—221° (dec.) was obtained as a purple powder from benzene. *Anal.* Calcd for $C_{27}H_{24}N_2O_4$: C, 73.62; H, 5.49; N, 6.36. Found: C, 73.87; H, 5.47; N, 6.66.

Compound II (150 mg) was dissolved in AcOH (8 ml), and treated with 30% $H_2O_2$ (15 ml) for 24 hr at room temperature. The reaction mixture was diluted with $H_2O$, and extracted with AcOEt. The AcOEt extract was evaporated to dryness and the residue was treated with boiling petr. benzin. The soluble fraction was separated by preparative TLC (acid-washed silica gel, *n*-hexane–AcOEt, 1: 1). The main band of the TLC gave colorless needles (III), mp 82—83°, from petr. benzin (yield, 23 mg). *Anal.* Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.25; H, 7.34; N, 5.88. MS *m/e*: 235 ($M^+$), 179, 161, 137. PMR ($CCl_4$) $\delta$: 0.97 (6H, d, $J$=2.8 Hz, $2CH_3$), 1.50—2.00 (3H, m, $CH_2$ and CH), 2.45 (2H, t, $J$=3.6 Hz, $-CH_2CO$) (these signals showed the presence of $-CO-CH_2CH_2CH(CH_3)_2$), 6.97 (1H, ddd, $J$=7.2, *ca.* 7.2, 1.0, aromatic H), 7.46 (1H, ddd, $J$=8.0, *ca.* 7.2, 1.6 Hz, aromatic H), 7.99 (1H, dd, $J$=7.2, 1.6 Hz, aromatic H), 8.65 (1H, dd, $J$=8.0, 1.0 Hz, aromatic H), 10.35 and 11.07 (each 1H, disappeared in $D_2O$).

From these results, Compound III was identified as N-(4,4-dimethylbutyroyl)anthranilic acid.

Compound III (15 mg) was refluxed with 10% HCl (5 ml) for 4 hr under an $N_2$ atmosphere. The solution was neutralized to pH 6.0 and extracted with AcOEt. The AcOEt was evaporated off and the residue was crystallized from *n*-hexane to provide colorless needles, mp 142—144° (5 mg). It was identified as anthranilic acid by IR spectroscopic comparison and mixed melting point determination.

Compound I (40 mg) was also oxidized with 30% $H_2O_2$ (5 ml) in 0.1 N NaOH (10 ml) for 25 min at room temperature. The reaction mixture was acidified and extracted with ether. The ether extract was purified by preparative TLC (acid-washed silica gel, $CHCl_3$–AcOEt, 2: 1). From the upper band, 2-(3,3-dimethylallyl)indole-3-carboxylic acid (IV), mp 173—174° was obtained (1.5 mg) as colorless needles from petr. benzin. *Anal.* Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 72.92; H, 6.43; N, 5.93. MS *m/e*: 229.110. UV $\lambda_{max}^{EtOH}$ nm: 217, 258, 288. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3360, 1650. PMR (acetone-$d_6$) $\delta$: 1.76 (6H, s, $2CH_3$), 3.96 (2H, d, $J$=6.8 Hz, $CH_2$), 5.45 (1H, t, $J$=6.8 Hz, –CH=), 7.04—7.18 (2H, m, aromatic H), 7.73 (1H, m, aromatic H), 8.05 (1H, m, aromatic H), 10.60 (1H, bs, NH).

From the lower band of the TLC, indole-3-carboxylic acid was obtained.

**Demethylation of AQ-B-1 with HCl in AcOH ($H_2O$ Addition to the 3,3-Dimethylallyl Group and Cyclization)**——AQ-B-1 (300 mg) was dissolved in 8% HCl (9 ml) and AcOH (15 ml), and the solution was refluxed for 15 min. The reaction mixture was poured into $H_2O$, and the resulting precipitate was dissolved in ether. The ether solution was extracted with 10% $NaHCO_3$ and then with 10% $Na_2CO_3$. The $NaHCO_3$ solution was acidified and the resulting precipitate was crystallized from MeOH as greenish-purple needles (VII), mp 206—208° (yield, 68 mg, 23%). VII was presumed to be a hydrate of demethyl AQ-B-1 on the basis of its physical properties. *Anal.* Calcd for $C_{32}H_{32}N_2O_5$: C, 73.26; H, 6.15; N, 5.34. Found: C, 72.92. H; 6.07, N; 5.17. MS *m/e*: 524 ($M^+$), 506 ($-H_2O$), 438. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3500, 3400, 3325 (OH and $NH_2$), 1630 (quinone). PMR (acetone-$d_6$) $\delta$: 1.30 (6H, s, $2CH_3$), 1.53 (6H, s, $2CH_3$), 1.90 (2H, $A_2B_2$-type, $CH_2$), 3.1 (2H, $A_2B_2$-type, $CH_2$), 4.80 (1H, dd, $J$=10, 1.6 Hz, vinyl H), 4.90 (1H, dd, $J$=17.6, 1.6 Hz, vinyl H), 6.7—7.5 (8H, m, aromatic H), 9.0 (2H, bs, 2OH), 9.83 (1H, bs, NH), 10.33 (1H, bs, NH).

The $Na_2CO_3$ solution was acidified and the resulting precipitate was purified by silica gel chromatography (benzene–AcOEt, 10: 1). The first greenish-purple eluate was crystallized from MeOH to give deep-purple needles (X), mp 242—243° (yield, 30 mg, 10%). Compound X was identified as cyclized demethyl AQ-B-1 from its PMR spectrum. *Anal.* Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87; H, 5.97; N, 5.53. Found: C, 76.05; H, 5.88; N, 5.38. MS *m/e*: 506 ($M^+$), 438, 409. PMR (DMSO-$d_6$) $\delta$: 1.43 (6H, s, $2CH_3$), 1.56 (6H, s, $2CH_3$), 2.06 (2H, bt, $J$=6 Hz, $CH_2$), 3.03 (2H, bt, $J$=6 Hz, $CH_2$), 4.97 (1H, dd, $J$=10.5, 1.6 Hz, vinyl H), 5.10 (1H, dd, $J$=17.0, 1.6 Hz, vinyl H), 6.12 (1H, dd, $J$=17.0, 10.5 Hz, –CH=), 6.90—7.46 (7H, m, aromatic H), 7.78 (1H, s, 2-CH of indole), 10.6 (2H, bs, 2OH), 10.92 (1H, s, NH).

The second purple eluate was crystallized from benzene as deep-purple prisms (demethyl AQ-B-1), mp 199—201° (yield, 61 mg, 20%). *Anal.* Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87; H, 5.97; N, 5.53. Found: C, 76.07; H, 5.93; N, 5.66.

AQ-C-2 gave a hydrated compound (IX), mp 251—252° (yield, 28%), Compound XI, mp 259—261° (yield, 14%), and demethyl AQ-C-2, mp above 300° (yield, 33%) on treatment with HCl in AcOH under the conditions used for AQ-B-1.

AQ-B-2 gave, under the same conditions, the hydrate (VIII), mp 236—238° (yield, 42%) and demethyl AQ-B-2, mp 192—195° (yield, 24%), but no cyclized derivative was obtained.

**Formation of Cyclized Demethyl AQ-B-1 (XI) from the Hydrate of Demethyl AQ-B-1 (VII)**——Compound VII (170 mg) was dissolved in AcOH (15 ml) and 8% HCl (9 ml), and the solution was heated on a boiling water bath. The reaction mixture was poured into $H_2O$, and the resulting precipitate was chromatographed on silica gel (benzene–AcOEt, 19: 1). From the first eluate, the cyclic compound (X), mp 242—243°, was isolated as greenish-purple needles from MeOH (66 mg). The second fraction gave demethyl AQ-B-1, mp 206—208° as purple prisms from benzene (15 mg). The starting material (49 mg) was recovered by elution with benzene–AcOEt (5: 1).

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## References and Notes

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9) All melting points are uncorrected.
10) Oxalic acid-treated silica gel (see Part IV of this series[1]) was used in all the chromatographic procedures in this work.