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Studies on the Metabolic Products of *Aspergillus terreus*. I. Metabolites of the Strain IFO 6123

Yuzuru Yamamoto, Ken-ichi Nishimura, and Noriki Kiriyama

Faculty of Pharmaceutical Sciences, Kanazawa University[1]

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The metabolites of *Aspergillus terreus* IFO 6123 were studied. 3-Methylorsellinic acid, 4-O-demethylbarbatic acid and a new metabolite named asterriquinone were isolated. The chemical structure of asterriquinone was established as 2,5-bis-[N-(1″,1″-dimethyl-2″-propenyl)-indoyl-3′]-3,6-dihydroxy-1,4-benzoquinone by chemical and physical experimental data. The incorporation experiments using ^{14}C-labeled compounds showed that asterriquinone is biosynthesized from tryptophan and isopentenyl unit derived from mevalonate.

In the course of the studies on the biosynthesis of the metabolites of *Aspergillus fumigatus* DH 413, it was shown that many hydroaromatic compounds were present in the metabolic pathway.[2] In order to confirm the roll of these hydroaromatic metabolites including epoxy compound, several strain of *Aspergillus terreus* were investigated, and meanwhile many new compounds were isolated. This paper deals with the metabolites of the strain IFO 6123, one of which was a new compound named asterriquinone.

Based on the results of preliminary experiments on the growth of mycelium, changes in pH, glucose content, and optical density (284 mμ) of the culture medium, this fungus was surface-cultured on a glucose-malt extract-polypeptone medium at 27° for 14 days.

The culture medium was concentrated under reduced pressure and then extracted with ethylacetate. When the ethylacetate solution was concentrated, terrein,[3] mp 127° was precipitated as colorless crystals. The mother liquor was chromatographed on a column of oxalic acid-precoated silicagel (see experimental) (solvent: benzene–ethylacetate, 9:1) and 3,6-dihydroxytoluquinone,[4] mp 179° was obtained as red prisms. The aqueous layer treated with ethylacetate was chromatographed on Dowex-1 as in the case of *Aspergillus fumigatus* DH 413, and a hydroaromatic compound, mp 189° was obtained as colorless prisms. These three metabolites were also obtained from *Aspergillus terreus* ATCC 12238, and will be reported in Part II of this series.

The slightly reddish-brown mycelium (yield: 70 g from 12 liters culture medium) was extracted in Soxhlet apparatus with petroleum ether, ether, and methanol successively. Ergosterol and mannitol were isolated from the petroleum ether and the methanol extracts, respectively. The ether extract was divided into acidic and neutral fractions with sodium bicarbonate. The acidic fraction was extracted with hot benzene, and compound I, mp 183—184° was obtained from the soluble part as colorless needles. Another compound (II), mp 175—176° was isolated from the sparingly soluble part as colorless needles. Compound III, mp 218—220° was obtained from the neutral fraction by chromatography on oxalic acid-precoated silicagel

1) Location: *Takaramachi 13-1, Kanazawa 920, Japan.*
2) Y. Yamamoto, K. Nitta, K. Tango, T. Saito, and M. Tsuchimuro, *Chem. Pharm. Bull.* (Tokyo), **13**, 935 (1965); Y. Yamamoto, K. Nitta, Y. Terashima, J. Ishikawa, and N. Watanabe, *ibid.*, **13**, 1009 (1965); Y. Yamamoto, K. Nitta, and A. Jinbo, *ibid.*, **15**, 427 (1967); Y. Yamamoto, M. Shinya, and Y. Oohata, *ibid.*, **18**, 561 (1970); Y. Yamamoto, K. Nitta, Y. Oohata, and T. Furukawa, *ibid.*, **20**, 931 (1972); Y. Yamamoto, T. Hirai, K. Okada, and K. Saito, *ibid.*, **22**, 83 (1974).
3) H. Raistrick and G. Smith, *Biochem. J.*, **29**, 606 (1935).
4) G. Pettersson, *Acta Chem. Scand.*, **17**, 1771 (1963).

and named asterriquinone. The yields were 5 mg (I), 45 mg (II), and 1.5 mg (III) from 1 g of mycelium.

A molecular formula $C_9H_{10}O_4$ was assigned for the compound I. The proton nuclear magnetic resonance (^1H NMR) spectrum showed the presence of two methyl groups (δ 2.04 and 2.50), one ring proton (6.30), two hydroxyl groups (7.6), and one carboxyl group (11.5). The infrared (IR) spectrum showed this carboxyl group (1640 cm^{-1}) was chelated with one of the two hydroxyl groups. These results combined with biogenetic point of view suggested the compound I is to be 3- or 5-methylorsellinic acid. The melting points of I and its methylester (mp 143—144°) corresponded to those of 3-methylorsellinic acid, mp 183—185°[5] and its methylester, mp 145°, but not to 5-methylorsellinic acid, mp 163°[6] (methylester, mp 126°). The final confirmation was made by comparison with an authentic sample of 3-methylorsellinic acid.[7]

Compound II has a molecular formula $C_{18}H_{18}O_7$. It afforded triacetate, mp 191° (ref,[8] 189—190°). By treatment with diazomethane for 10 min, it gave monomethylester (IV), mp 112° (ref,[8] 108—111°), but by longer treatment (overnight) it gave monomethylether of IV, mp 169°. These results showed two of the three hydroxyl groups were chelated with two carbonyl groups (1662 and 1637 cm^{-1}), one of which must be carboxyl group. ^1H NMR spectrum of the compound IV showed the existence of four methyl groups and two isolated ring protons (singlet) together with the functional groups mentioned above. The ultraviolet (UV) spectrum had maximum absorptions at 278 and 308 mμ. From these results, the compound II was assigned to be 4-O-demethylbarbatic acid, mp 176—177°, which is a metabolite of a lichen, *Ramalina subdecipiens* Stein[8]. According to the described method,[8] the compound II was cleaved to 3-methylorsellinic acid by treatment with concentrated sulfuric acid in 65% yield. Methylester of the compound II was also cleaved with concentrated sulfuric acid into both 3-methylorsellinic acid and its methylester. The compound II was completely coincided with the lichen metabolite in IR, NMR, and mass spectra. This is the first example of depside obtained from fungi.

A molecular formula $C_{32}H_{30}O_4N_2$ was established for the compound III (asterriquinone) by mass spectral and elementary analyses. The deep purple color ($\lambda_{max}^{CHCl_3}$, 298 and 508 mμ) was decolored by treatment with zinc powder in acetic acid (λ_{max}, 296 mμ), and the color was recovered by standing overnight. The IR spectrum of asterriquinone had absorptions at 3300 (OH), 1640, and 1620 cm^{-1} (quinone), and these peaks very resembled those of 3,6-dihydroxytoluquinone or 3,6-dihydroxy-4-methoxytoluquinone (spinulosin). Asterriquinone gave tetraacetate, $C_{40}H_{40}O_8N_2$, mp 257—259° (decomp.), by acetylation under the presence of zinc powder. By treatment with diazomethane it afforded dimethylether (V), $C_{34}H_{34}O_4N_2$,

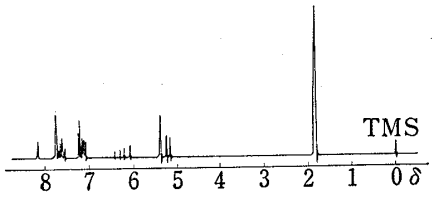


Fig. 1. ^1H NMR Spectrum of Asterriquinone (100 MHz, in $CDCl_3$)

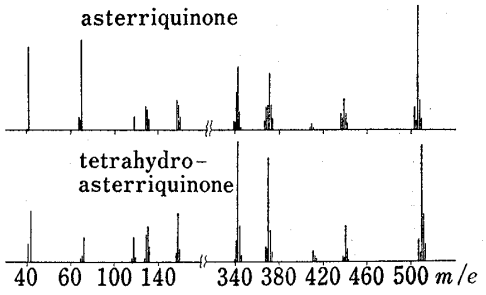


Fig. 2. Mass Spectra of Asterriquinone and Tetrahydroasterriquinone

5) S. Takenaka, N. Ojima, and S. Seto, *J. Chem. Soc. Commun.*, **1972**, 391.
6) R.F. Curtis, P.C. Harries, C.H. Hassall, and J.D. Levi, *Biochem. J.*, **90**, 43 (1964).
7) The sample was kindly supplied by Prof. S. Seto, Tohoku University.
8) S. Huneck, G. Follmann, and J. Santesson, *Z. Naturforsch.*, **23B**, 856 (1968).

mp 168°, and the methylether V gave diacetate (VI), $C_{38}H_{36}O_6N_2$, mp 212° by reductive acetylation.

The ^1H NMR spectrum of asterriquinone (Fig. 1) showed the peaks at δ 1.82 (s,[9] 4 CH_3), 5.24 (d,[9] J=18 Hz, 2H), 5.26 (d,[9] J=10 Hz, 2H), and 6.20 (dd,[9] J=10 and 18 Hz, 2H), together with 10 aromatic ring protons at 7.10—7.22 (m,[9] 4H), 7.50—7.70 (m,[9] 4H), and 7.71 (s,[9] 2H). The signal of two hydroxyl protons at δ 8.14 was confirmed by the addition of deuterium oxide. The ^1H NMR spectrum showed the presence of pairs of each group of protons and a symmetrical structure of the molecule. This was also well shown in the ^1H NMR spectrum of the tetraacetate in which four acetoxy methyl protons appeared as a single peak at δ 1.86. In the mass spectra, asterriquinone (M^+, *m/e* 506) and its tetraacetate (M^+, *m/e* 676) had fragmentation peaks at *m/e* 69 (C_5H_9), M^+-68, and M^+-136 (see Fig. 2). These results suggested the presence of two isopentenyl groups which contained vinyl groups.

When asterriquinone was hydrogenated with Pd/C in ethanol, it absorbed three moles of hydrogen to give a colorless solution, but the color was recovered by exposure in the air, and the resulted purple compound, mp 263—264°, corresponded to tetrahydro-derivative, $C_{32}H_{34}O_4N_2$. The mass spectrum of this compound (Fig. 2, M^+, *m/e* 510) had fragmentation peaks at *m/e* 71, 440 (M^+-70), and 370 (M^+-140) which corresponded to the peaks related to the isopentenyl groups of the parent compound. In the ^1H NMR spectrum of the tetrahydro compound, the coupled signals of CH_3 (δ 0.72, t,[9] J=8 Hz) and CH_2 (2.16, q,[9] J=8 Hz) were observed instead of the signals of vinyl groups in asterriquinone. The original signal of the methyl group at δ 1.76 remained singlet, so the isopentenyl group of asterriquinone must be 1,1-dimethyl-2-propenyl group.

From the results above it was shown that asterriquinone is a symmetrical dihydroxy-1,4-benzoquinone which is symmetrically substituted with two $C_{13}H_{14}N$ groups. And this substituent contains five ring protons and 1,1-dimethyl-2-propenyl group as shown in Fig. 3.

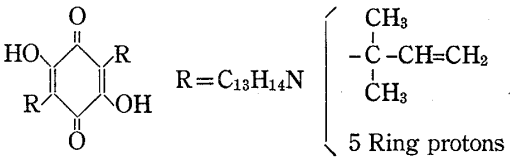


Fig. 3

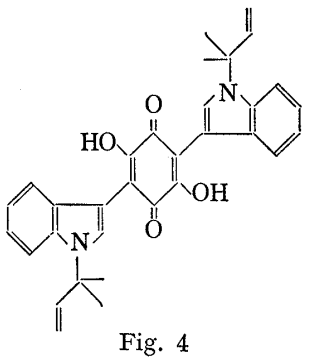


Fig. 4

To determine the structure of the substituted group R, asterriquinone was oxidatively cleaved with hydrogen peroxide in dilute alkali at room temperature. The reaction mixture was acidified and the resulted precipitate was crystallized from petroleum benzin as colorless prisms (VII), mp 160—162° (decomp.). A molecular formula $C_{14}H_{15}O_2N$ was assigned for the compound VII. The ^1H NMR spectrum contained all the signals of the residue R (14 protons) together with an additional signal of carboxyl proton at δ 10.60. No absorption of NH-streching in IR spectrum, positive Ehrlich reaction (purple-red), UV spectrum (λ_{max}^{EtOH}, 288 mμ, log ε=4.10), the presence of five ring protons, and their decoupling data, all suggested the compound VII is an N-substituted indole carboxylic acid. The IR absorption of carbonyl group at 1650 cm^{-1} coincided well with that of indole-3-carboxylic acid (1640 cm^{-1}) and the ^{13}C NMR signal at δ_C 60.1 in the compound VII (59.6 in asterriquinone) could be assigned to the quartary carbon of the isopentenyl group attached to the nitrogen atom. From these

9) Abbreviation: s, singlet; d, doublet; t, triplet; dd, double doublet; q, quartet; m, multiplet.

results, the structure of the compound VII was proposed as N-(1′,1′-dimethyl-2′-propenyl)-indole-3-carboxylic acid.

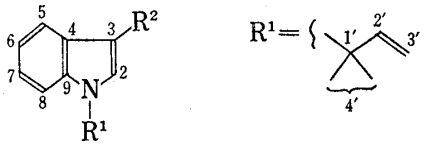
Diphenyldihydroxybenzoquinone (terphenyl quinone) such as polyporic acid is easily cleaved with hydrogen peroxide at room temperature,[10] and this is also the case in asterriquinone. Finally, asterriquinone was determined as 2,5-bis-[N-(1″,1″-dimethyl-2″-propenyl)-indoyl-3′]-3,6-dihydroxy-1,4-benzoquinone as shown in Fig. 4.

^{13}C NMR spectra of asterriquinone and several related compounds are listed in Table I and II. These spectra also elucidated the structure of asterriquinone. At room temperature, signals of the carbonyl and hydroxy-bearing carbons of asterriquinone were not observed, but when measured at −75°, reasonable signals appeared at δ_c 182.6 (C=O) and 149.7 (C–OH). This phenomenon can be attributed to rapid interconversion between two equivalent tautomeric forms of the hydroxy quinone moiety. Similar phenomena were also observed in 3,6-dihydroxy-toluquinone and spinulosin as shown in Table II. Dimethylether of asterriquinone (V) showed the signals at δ_c 184.0 (C=O) and 153.2 (C–OCH_3) normally.

Table I. ^{13}C NMR Spectra of Indole Moiety (δ_c ppm)

	Carbons in indole part						Carbons of substituted group					
							R^1				R^2	
	2	3	4	5,6,7	8	9	1′	2′	3′	4′	COOH	CH_3
Asterriquinone[a]	128.2	102.4	128.0	121.1 122.0 119.1	113.8	135.4	59.6	143.7	110.9	27.9	—	—
Compound VII[a]	133.3	105.8	128.1	122.6 121.8 121.8	114.4	136.0	60.1	142.8	114.4	27.9	170.9	—
3-Methylindole[b]	121.6	110.9	128.0	118.6 121.6 118.9	110.9	136.0	—	—	—	—	—	9.4
Indole[b]	124.1	102.1	127.6	121.7	111.0	135.5	—	—	—	—	—	—

a) solvent, $CDCl_3$; temperature, 23°
b) solvent, $CDCl_3$; temperature, 20° (cited from "Carbon-13 NMR Spectra," eddited by L.F. Johnson and W.C. Jankowski, John Wiley and Sons Inc., 1972)



Echinuline,[11] neoechinuline,[12] brevianamide,[13] verruchlogen,[14] fumitremorgin A and B,[15] desoxybrevianamide E,[16] and cochliodinol,[17] *etc.* are known as tryptophan derived fungal metabolites which are substituted with isopentenyl group. But asterriquinone is the first example of the compound having reverse isopentenyl group on nitrogen atom.

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15) M. Yamazaki, K. Sasago, and K. Miyaki, *J. Chem. Soc. Commun.*, **1974**, 408.
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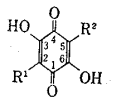
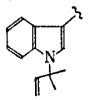
Table II. ^{13}C NMR Spectra of Quinone Moiety (δ_c ppm)

	Solvent	Temperature	Carbons of quinone ring				Substituted group	
			1, 4	3, 6	2	5	CH_3	OCH_3
Asterriquinone	$CDCl_3$	23°	×[a]	×[a]	113.9	113.9	—	—
		−75°	182.6	149.7	113.9	113.9	—	—
Compound V	$CDCl_3$	23°	184.0	153.2	113.8	113.8	—	60.4
3,6-Dihydroxy-toluquinone	THF[b]+	23°	×	×	113.2	103.6	7.5	—
	THF-D_8(5:1)	−100°	186.1 194.8	144.2 160.5	118.6	112.8	7.7	—
Spinulosin	$CDCl_3$	23°	×	×	111.7	135.3	7.5	59.7
	THF+	23°	not clear	not clear	104.7	112.1	7.5	60.1
	THF-D_8(1:1)	−70°	186.6 194.8	144.8 159.9	109.9	118.1	7.6	60.0

a) not observed
b) tetrahydrofuran



asterriquinone: $R^1=R^2=$ 

compound V: dimethylether of asterriquinone
3,6-dihydroxytoluquinone: $R^1=CH_3$, $R^2=H$
spinulosin: $R^1=CH_3$, $R^2=OCH_3$

As suggested by the chemical structure, asterriquinone presumed to be biosynthesized from tryptophan and isopentenyl unit derived from mevalonic acid. The yield of asterriquinone depended on the nitrogen and tryptophan content in the culture medium. Thus, a maximum yield was observed when polypeptone content was 4 g/liter (five times more production than in the usual condition, 1 g/liter). However, excess amount of tryptophan seemed to cause inhibition.

To confirm the participation of tryptophan and mevalonate, administration experiments were attempted by using ^{14}C-compounds. The results are shown in Table III. When DL-tryptophan-3-^{14}C was administered to the fungus (5th day's administration and further

Table III. Distribution of Radioactivity in the Culture and in the Molecule of Asterriquinone

		Amount	Total radio-activity dpm	Incor-poration ratio %	Specific radio-activity dpm/mM
DL-Tryptophan-3-^{14}C	^{14}C used		98.2×10^6		
	Mycelium	17.3 g	64.7×10^6	65.8	
	Culture medium	2.4 L	29.9×10^6	30.4	
	Respiratory CO_2		0.8×10^6	0.8	
	Asterriquinone	58.9 mg	13.4×10^6	13.6	115×10^6
DL-Mevalonate-2-^{14}C	^{14}C used		19.7×10^6		
	Mycelium	17.6 g	2.4×10^6	12.2	
	Culture medium	2.4 L	17.9×10^6	90.9	
	Respiratory CO_2		8.0×10^4	0.4	
	Asterriquinone	65.8 mg	1.4×10^4	0.07	10.8×10^4

Degradation of Asterriquinone Derived from Tryptophan-3-^{14}C

Asterriquinone —(H_2O_2)→ Compound VII —(NaN_3/H_2SO_4)→ CO_2

Asterriquinone	Compound VII	CO_2
1.19×10^6 dpm/mM	0.59×10^6 dpm/mM	0.53×10^6 dpm/mM
(100%)	(90%)	(89%)

7 days' cultivation), the radioactivity was incorporated well into asterriquinone (13.6%). The radioactive asterriquinone was degraded with hydrogen peroxide, and almost all radioactivity (99%) was detected in the resulted N-substituted indole carboxylic acid (VII). It was further degraded by the Schmidt reaction, and 90% of the radioactivity was found in the liberated carbon dioxide. This result showed that the carbon skeleton of tryptophan was incorporated into asterriquinone without any modification. Mevalonate was also incorporated into asterriquinone, although incorporation ratio was unexpectedly low (0.07%) and more than 90% of the radioactivity remained in the culture medium. This result might due to inadequate administration conditions.

Asterriquinone showed anti-tumor activity against Ehrlich ascites carcinoma in mice, lymphatic leukemia L 1210 in mice, and ascites hepatoma AH 109A in rats, but it had little anti-bacterial effect. On these aspects, it will be reported in the near future.

Experimental[18]

Cultural Conditions——*Aspergillus terreus* IFO 6123 was cultivated stationarily in 500 ml-Roux flasks containing 200 ml of the culture medium (glucose, 20 g; malt extract (Difco), 20 g; polypeptone (Daigoeiyo), 1 g; tap water, 1 liter). After cultivation at 27° for 14—16 days, the mycelium was harvested by filtration, washed with H_2O, and warm-air-dried.

Isolation of Metabolites from Mycelium——The dried mycelium was extracted in Soxhlet apparatus with petr. ether, ether, and MeOH, successively. Ergosterol was obtained from the petr. ether fraction (yield, 2 mg/g dry mycelium), and mannitol was isolated from the MeOH fraction (30 mg/g mycelium). The ether extract was divided into acidic and neutral fractions by treatment with 5% $NaHCO_3$ solution. The acidic fraction was extracted with benzene by refluxing. The soluble part in benzene was adsorbed on a column of silicagel (Mallinckrodt, Silic AR CC-4) and eluted with *n*-hexane–AcOEt (8: 2, v/v). The first effluent was recrystallized from aqueous MeOH as colorless needles (I), mp 183—184° (ref.[5] 183—185°). *Anal.* Calcd. for $C_9H_{10}O_4$: C, 59.33; H, 5.53. Found: C, 59.56; H, 5.54. UV λ_{max}^{EtOH} μm (log ε): 218 (4.42), 266 (4.10), 302 (3.57). IR ν_{max}^{KBr} cm^{-1}: 3400, 3000, 1640. ^1H NMR ($CDCl_3$) δ: 2.04 (s, CH_3), 2.50 (s, CH_3), 6.30 (s, 1H), 7.6 (broad s, 2 OH), 11.50 (broad s, COOH). Mass Spectrum *m/e*: 182 (M^+), 164, 138, 136.

The sparingly soluble part in hot benzene was recrystallized from aqueous MeOH as colorless needles (II), mp 175—176° (decomp.) (ref,[8] 175—177°). *Anal.* Calcd. for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found: C, 62.38; H, 5.14. UV λ_{max}^{EtOH} mμ (log ε): 278 (4.42), 308 (4.06). IR ν_{max}^{KBr} cm^{-1}: 3400, 3200—3100, 1662, 1637. ^1H NMR (DMSO-d_6) δ: 2.01 (s, 1H), 8.3 (bs, OH), 10.2 (bs, OH), 11.1 (bs, COOH). Mass Spectrum *m/e*: 346 (M^+), 182, 165, 164, 138, 137, 136, 123.

The neutral fraction obtained from the ether extract was applied on a column of oxalic acid-precoated silicagel,[19] and eluted with benzene. The purple effluent was evaporated and the resulted residue was crystallized from MeOH as dark purple needles (III, asterriquinone), mp 218—220° (decomp.). *Anal.* Calcd. for $C_{32}H_{30}O_4N_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 76.03; H, 5.92; N, 5.48. UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 298 (4.51), 508 (3.74). IR ν_{max}^{KBr} cm^{-1}: 3300, 1640, 1620. 1458, 996, 912 (vinyl). ^1H NMR ($CDCl_3$) δ: 1.82 (s, $4CH_3$), 5.24 (d, J=18, 2H), 5.26 (d, J=10, 2H), 6.20 (dd, J=10, 18, 2H), 7.10—7.22 (m, 4H), 7.50—7.70 (m, 4H), 7.71 (s, 2H), 8.14 (s, 2 OH). Mass Spectrum *m/e*: 506 (M^+), 438, 370, 342, 341, 313, 285, 257, 256, 155, 129, 128, 117, 69. $[\alpha]_D$=0° (c=0.25, EtOH).

Methylester of I——Colorless needles, mp 143—144°. *Anal.* Calcd. for $C_{10}H_{12}O_4$: C, 61.21; H, 6.17. Found: C, 61.43; H, 6.05. Mass Spectrum *m/e*: 196 (M^+), 178, 150, 107, 77.

Methylester of II——Colorless needles (IV), mp 108—112°. *Anal.* Calcd. for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 63.34; H, 5.70. Mass Spectrum *m/e*: 360 (M^+), 196, 165, 164, 136.

Monomethylether of IV——Compound II was treated with etherial diazomethane overnight. The resulted compound was crystallized from MeOH as colorless needles, mp 167—169°. *Anal.* Calcd. for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.27; H, 5.85.

Acetate of II——To the solution of II (500 mg) in Ac_2O (5 ml), two drops of conc. H_2SO_4 was added and kept at room temperature for 20 hr. The reaction mixture was poured into ice water and the resulted precipitate was crystallized from MeOH as colorless prisms, mp 190—191° (yield, 190 mg). *Anal.* Calcd. for $C_{24}H_{24}O_{10}$ (triacetate): C, 61.01; H, 5.12. Found: C, 60.99; H, 5.14. IR ν_{max}^{KBr} cm^{-1}: 1780, 1690.

Cleavage of II with Conc. H_2SO_4——II (100 mg) was dissolved in conc. H_2SO_4 (2 ml) and kept at 0° for 20 min. The reaction mixture was poured into ice water, and the resulted precipitate was crystallized from

18) All melting points are not corrected.

19) Silicagel (for chromatography, Kanto Chemical Co. Ltd.) was suspended in 0.1 M oxalic acid overnight, filtered, washed with H_2O and dried in an oven at 100°.

aqueous MeOH as colorless needles, mp 183—184° (56 mg). *Anal.* Calcd. for $C_9H_{10}O_4$: C, 59.33; H, 5.53. Found: C, 59.48; H, 5.43. It was identified with 3-methylorsellinic acid by IR spectrum and mixed mp.

Cleavage of IV——IV (300 mg) was treated by the same method in the cleavage of II. The resulted precipitate was extracted with ether, and the ether extract was divided into neutral and acidic fractions by treatment with 5% $NaHCO_3$ solution. From the neutral fraction, 3-methylorsellinic acid methyl ester, mp 143—144° was obtained (103 mg), and 3-methylorsellinic acid was isolated from the acidic fraction (125 mg).

Dimethylether of Asterriquinone——To the solution of 50 mg of asterriquinone in ether, excess etherial diazomethane was added and reacted for 10 min. After evaporation, the residue was crystallized from EtOH as purple needles, (V), mp 168° (42 mg). *Anal.* Calcd. for $C_{34}H_{34}O_4N_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.41; H, 6.32; N, 5.28. UV λ_{max}^{EtOH} mμ: 289, 295, 486. IR ν_{max}^{KBr} cm^{-1}: 1652, 1586, 1460, 1380, 1370. ^1H NMR ($CDCl_3$) δ: 1.86 (s, 4 CH_3), 3.80 (s, 2 OCH_3), 5.26 (d, $J=16$ Hz, 2H), 5.29 (d, $J=11$ Hz, 2H), 6.26 (dd, $J=11$, 16 Hz, 2H), 7.10—7.28 (m, 4H), 7.52—7.78 (m, 6H). Mass Spectrum *m/e*: 534 (M^+), 466, 423, 398, 355, 255, 69.

Reductive Acetylation of Asterriquinone——Asterriquinone (50 mg) was refluxed with Ac_2O under the presence of fused AcONa (200 mg) and zinc powder (200 mg) for 1 hr. The reaction mixture was poured into H_2O. The resulted precipitate was crystallized from AcOEt as colorless prisms, mp 257—259° (decomp.) (22.5 mg). *Anal.* Calcd. for $C_{40}H_{40}O_8N_2$ (tetraacetate): C, 70.99; H, 5.96; N, 4.41. Found: C, 70.96; H, 5.74; N, 4.15. UV $\lambda_{max}^{CHCl_3}$ mμ (log ε): 310 (4.40). IR ν_{max}^{KBr} cm^{-1}: 1775, 1615. ^1H NMR ($CDCl_3$) δ: 1.80 (s, 4 CH_3), 1.86 (s, 4 $COCH_3$), 5.17 (d, $J=17$ Hz, 2H), 5.31 (d, $J=11$ Hz, 2H), 6.20 (dd, $J=11$, 17 Hz, 2H), 7.12—7.80 (m, 10 H). Mass Spectrum *m/e*: 676 (M^+), 634, 592, 524, 508, 481, 440, 372, 371, 342, 158, 69.

Reductive Acetylation of V——V (50 mg) was treated by the same method described above, and the resulted acetate was crystallized from MeOH as colorless needles, (VI), mp 212° (42.5 mg). *Anal.* Calcd. for $C_{38}H_{40}O_6N_2$ (diacetate): C, 73.52; H, 6.50; N, 4.51. Found: C, 73.26; H, 6.37; N, 4.38. UV λ_{max}^{EtOH} mμ: 225, 305. IR ν_{max}^{KBr} cm^{-1}: 1768, 1610. ^1H NMR ($CDCl_3$) δ: 1.96 (s, 4 CH_3), 1.84 (s, 2 $COCH_3$), 3.36 (s, 2 OCH_3), 5.18 (d, $J=18$ Hz, 2H), 5.28 (d, $J=11$ Hz, 2H), 6.24 (dd, $J=11$, 18 Hz, 2H), 7.08—7.28 (m, 4H), 7.44—7.72 (m, 6H). Mass Spectrum *m/e*: 620 (M^+), 578, 552, 535, 510, 509, 468, 400, 169, 131, 119, 69.

Catalytic Hydrogenation of Asterriquinone——Asterriquinone (100 mg) was dissolved in EtOH, 50 mg of 5% Pd/C was added and shaken under the atmosphere of hydrogen. Within 30 min 3 moles of hydrogen was absorbed and the solution became colorless. Purple color was regenerated during filtration for removing the catalyst. The solvent was evaporated and the residue was recrystallized from MeOH as deep purple needles, mp 263—264° (22 mg). *Anal.* Calcd. for $C_{32}H_{34}O_4N_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.29; H, 6.50; N, 5.37. UV λ_{max}^{EtOH} mμ: 295, 466. IR ν_{max}^{KBr} cm^{-1}: 3300, 1630, 1530. ^1H NMR ($CDCl_3$) δ: 0.72 (t, $J=8$ Hz, 2 CH_3), 2.16 (q, $J=8$ Hz, 2 CH_2), 1.76 (s, 4 CH_3), 7.12—7.28 (m, 4H), 7.60—7.76 (m, 6H), 8.12 (s, 2 OH). Mass Spectrum *m/e*: 510 (M^+), 440, 370, 342, 256, 157, 129, 117, 71.

Oxidative Cleavage of Asterriquinone with H_2O_2——Finely powdered asterriquinone (100 mg) was dissolved in 0.1 N NaOH (20 ml) and 10 ml of 30% H_2O_2 was added. After standing at room temperature for 5 hr, pale yellow reaction mixture was acidified with HCl. The resulted slightly gray precipitate was filtered, washed with H_2O, and recrystallized from petr. benzin as colorless prisms, (VII), mp 160—162° (33 mg). *Anal.* Calcd. for $C_{14}H_{15}O_2N$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.40; H, 6.55; N, 6.07. UV λ_{max}^{EtOH} mμ (log ε): 218 (4.49), 288 (4.10). IR ν_{max}^{KBr} cm^{-1}: 1650, 1610, 1530, 1370, 1360. ^1H NMR ($CDCl_3$) δ: 1.82 (s, 2 CH_3), 5.20 (d, $J=18$ Hz, 1H), 5.28 (d, $J=11$ Hz, 1H), 6.20 (dd, $J=11$, 18 Hz, 1H), 7.26 (m, 2H), 7.59 (m, 1H), 8.16 (s, 1H), 8.30 (m, 1H), 10.60 (broad s, COOH). Mass Spectrum *m/e*: 229 (M^+), 185, 161, 144, 117, 69.

Incorporation Experiments with ^{14}C-Labeled Compounds——DL-Tryptophan-3-^{14}C (New England Nuclear) and DL-mevalonic acid-2-^{14}C (The Radiochemical Centre) were used. Each labeled compound was administered on the 5th day of the cultivation. After further 7 days' cultivation, the radioactivity of the culture medium, mycelium (by combustion), and liberated CO_2 were assayed in a liquid scintillation spectrometer (Packard Tri-Carb, Model 3320; 7 g PPO, 0.3 g dimethyl POPOP, 100 g naphthalein in 1 liter dioxane as the scintillator). CO_2 was collected as $BaCO_3$ and suspended in the scintillator with Cab-O-Sil before assay. The radioactive asterriquinone was diluted with non-labeled sample, and degraded to determine the distribution of radioactivity in the molecule.

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