FOUR NEW METABOLITES OF *ASPERGILLUS TERREUS*

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Four new metabolites (**1**-**4**) were isolated from mycelium of *Aspergillus terreus* IFO 6123 producing asterriquinone (ARQ). The structures of **1** and **2** were shown to be 3,6-bis[1-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-furo[3,2-*b*]furan-2,5-dione (asterridinone) and 2,5-bis[1-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-3-acetoxy-6-hydroxy-2,5-cyclohexadiene-1,4-dione (ARQ monoacetate) , respectively, by the chemical and spectral data. Compounds **3** and **4** were identified with known asterriquinone isomers, 2-[1-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-5-[2-(3-methyl-2-butenyl)-1*H*-indol-3-yl]-3,6-dihydroxy-2,5-cyclohexadiene-1,4-dione (isoARQ) and 2,5-bis[2-(3-methyl-2-butenyl)-1*H*-indol-3-yl]-3,6-dihydroxy-2,5-cyclohexadiene-1,4-dione (neoARQ), respectively.

KEYWORDS *Aspergillus terreus* IFO 6123; asterriquinone monoacetate; asterridinone; isoasterriquinone; neoasterriquinone; fungal metabolite

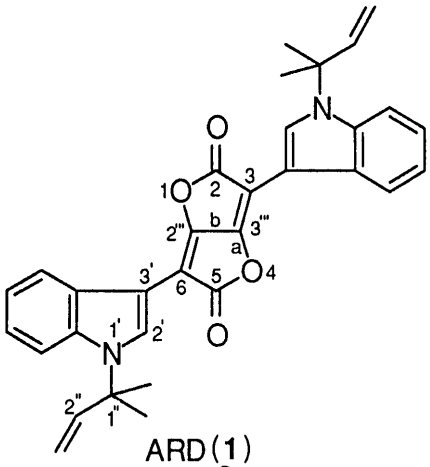
An antitumor agent, asterriquinone (ARQ), is known to be a metabolite isolated from mycelium of *Aspergillus terreus* IFO 6123.[1] This paper deals with four additional new metabolites of the fungus.

This fungus was cultivated stationarily on the modified malt extract medium (malt extract, 20g; glucose, 20g; polypeptone, 5g; L-tryptophan, 0.11g; tap water, 1 l) at 27°C for 14 days. The dry mycelium (yield, 90g from 12 liters culture medium) was extracted in a Soxhlet extractor with petroleum ether and dichloromethane, successively. Each extract was evaporated, dissolved in *n*-hexane-dichloromethane (1:1), and chromatographed on a column of oxalic acid-impregnated silica gel with gradiently changed *n*-hexane-dichloromethane (1:1→0:1). The petroleum ether extract gave compound **1** (5 mg), ARQ (40 mg), and compound **2** (30 mg). From the dichloromethane extract, ARQ (690 mg), **2** (6 mg), compound **3** (32 mg), and compound **4** (44 mg) were isolated.

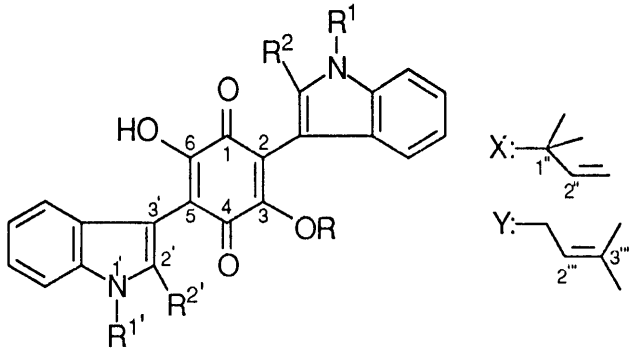
Compound **1**, named asterridinone (ARD), was obtained as orange needles from MeOH, mp 261-263°C (dec.), $[\alpha]_D \pm 0°$ (c = 0.25, $CHCl_3$, 25°C), UV λ_{max} (EtOH) nm (log ε): 228 (4.39), 271 (3.80), 292 (3.88), 479 (4.25), and its formula, $C_{32}H_{28}N_2O_4$, was confirmed by the high-resolution mass spectrum (found; 504.2049, calcd; 504.2069). The IR spectrum (Nujol) showed absorption at 1810 and 1784 cm^{-1}, indicating the presence of the lactone group, but no absorption due to hydroxy group was observed. The ^1H-NMR spectrum ($CDCl_3$) revealed the presence of two 1,1-dimethyl-2-propenyl groups [δ 1.85 (12H, s),

δ 5.23 (2H, d, *J*=17.6 Hz), δ 5.29 (2H, d, *J*=10.8 Hz), δ 6.17 (2H, dd, *J*=10.8, 17.6 Hz)] and 10 aromatic ring protons[δ 7.21-7.30 (4H, m), δ 7.58 (2H, d, *J*=8.0 Hz), δ 8.37 (2H, s)]. This spectrum showed the presence of pairs of each group of protons and a symmetrical structure of the molecule. From these results, it was suggested that the chemical structure of ARD was 3,6-bis[1-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-furo[3,2-*b*]furan-2,5-dione. Finally, the structure of **1** was confirmed by transformation of ARQ to **1** by oxidation with dimethylsulfoxide in the presence of acetic anhydride.[2] As a similar compound, cochliodinone, metabolite of *Chaetomium cochliodes*, had been reported.[3]

Compound **2** was obtained as blue purple needles from *n*-hexane-dichloromethane, mp 172-174°C (dec.), $[\alpha]_D \pm 0°$ (c = 0.25, $CHCl_3$, 25°C), UV λ_{max} (EtOH) nm (log ε): 225 (4.66), 292 (4.44), 487 (3.81), and its formula, $C_{34}H_{32}N_2O_5$, was established by the high-resolution mass spectrum (found; 548.2311, calcd; 548.2331). The IR spectrum (KBr) showed aromatic hydroxy group absorption at 3404 cm^{-1}, acetoxy group at 1764 cm^{-1}, and quinone group at 1644 and 1610 cm^{-1}. The ^{1}H-NMR spectrum ($CDCl_3$) indicated the presence of two unequivalent 1,1-dimethyl-2-propenyl groups[δ 1.82 (6H, s), δ 5.25 (1H, d, *J*=17.6 Hz), δ 5.27 (1H, d, *J*=10.4 Hz), δ 6.20 (1H, dd, *J*=10.4, 17.6 Hz); δ 1.84 (6H, s), δ 5.27 (1H, d, *J*=17.6 Hz), δ 5.30 (1H, d, *J*=10.4 Hz), δ 6.20 (1H, dd, *J*=10.4, 17.6 Hz)], an acetoxy group[δ 2.15 (3H, s)], a hydroxy group [δ 7.47 (1H, s)], and 10 aromatic ring protons [δ 7.13-7.19 (4H, m), δ 7.55-7.65 (4H, m), δ 7.74 (1H, s), δ 7.76 (1H, s)]. Compound **2** was treated with ethanolic sodium hydroxide aqueous solution to give ARQ. From these results, the structure of **2** was determined as 2,5-bis[1-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-3-acetoxy-6-hydroxy-2,5-cyclohexadiene-1,4-dione (ARQ monoacetate).



ARD (**1**)



	R^1	R^2	$R^{1'}$	$R^{2'}$	R
ARQ monoacetate (**2**)	X	H	X	H	Ac
isoARQ (**3**)	X	H	H	Y	H
neoARQ (**4**)	H	Y	H	Y	H
ARQ	X	H	X	H	H

Compound **3** (isoARQ) was obtained as dark purple needles from *n*-hexane-dichloromethane, mp 150-151°C (dec.), $[\alpha]_D \pm 0°$ (c = 0.25, $CHCl_3$, 25°C), UV λ_{max} (EtOH) nm (logε): 225 (4.68), 282 (4.50), 288 (4.49), 477 (3.43), and has the same molecular formula, $C_{32}H_{30}N_2O_4$ [by the high-resolution mass spectrum (found; 506.2198, calcd; 506.2206)], as ARQ. The IR spectrum (KBr) showed aromatic hydroxy group absorption at 3420 cm^{-1}, NH group at 3316 cm^{-1}, and quinone group at 1634 cm^{-1}. The ^{1}H-NMR spectrum ($CDCl_3$) indicated the presence of a 1,1-dimethyl-2-propenyl group[δ 1.84 (6H, s), δ 5.27 (1H, d,

J=17.6 Hz), δ 5.28 (1H, d, J=10.6 Hz), δ 6.23 (1H, dd, J=10.6, 17.6 Hz)], a 3-methyl-2-butenyl group[δ1.77 (3H, d, J=1.1 Hz), δ1.83 (3H, d, J=1.1 Hz), δ3.46 (2H, d, J=7.3 Hz), δ5.42 (1H, t-like, J=7.3 Hz], 9 aromatic ring protons [δ 7.12-7.66 (8H, m), δ 7.75 (1H, s)], two hydroxy groups [δ 8.10 (2H, s)], and an NH group [δ 8.19 (1H, br s)]. From these results, the structure of **3** was suggested as 2-[1-(1,1-dimethyl-2-propenyl)-1*H*-indol-3-yl]-5-[2-(3-methyl-2-butenyl)-1*H*-indol-3-yl]-3,6-dihydroxy-2,5-cyclohexadiene-1,4-dione.

Compound **4** (neoARQ) was obtained as dark purple needles from *n*-hexane-dichloromethane, mp 192-193°C (dec.), $[\alpha]_D \pm 0°$ (c = 0.25, $CHCl_3$, 25°C), UV λ_{max} (EtOH) nm (logε): 226 (4.68), 282 (4.49), 288 (4.48), 485 (3.26), and has the same molecular formula, $C_{32}H_{30}N_2O_4$ [by the high-resolution mass spectrum (found; 506.2442, calcd; 506.2206)], as ARQ. The IR spectrum (KBr) showed aromatic hydroxy group absorption at 3408 cm^{-1}, NH group at 3356 cm^{-1}, and quinone group at 1632 cm^{-1}. The ^{1}H-NMR spectrum ($CDCl_3$) indicated the presence of two equivalent 3-methyl-2-butenyl groups[δ 1.77 (6H, s, J=1.1 Hz), δ 1.83 (6H, s, J=1.1 Hz), δ 3.47 (4H, d, J=7.3 Hz), δ 5.42 (2H, t-like, J=7.3 Hz)], 8 aromatic ring protons [δ 7.12-7.36 (8H, m)], two hydroxy groups [δ 8.07 (2H, s)], and two NH groups [δ 8.20 (2H, br s)]. From these results, the structure of **4** was suggested as 2,5-bis[2-(3-methyl-2-butenyl)-1*H*-indol-3-yl]-3,6-dihydroxy-2,5-cyclohexadiene-1,4-dione. Finally, the structures of **3** and **4** were confirmed by direct comparison with authentic samples (ref.[4] 164-165°C; 196-199°C), which were prepared by treatment of ARQ with HCl in AcOH.

Among these metabolites, ARD did not affect the growth of mouse leukemia P388 cells, but the others inhibited cell growth with similar potency of ARQ (IC_{50} = 1.28 ± 0.06 μM). More detailed biological activities of these metabolites are now under investigation.

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