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Antitumor Activity of Asterriquinones from *Aspergillus* Fungi. IV.[1] An Attempt to modify the Structure of Asterriquinones to increase the Activity

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An attempt was made to obtain more potent antitumor compounds by chemically modifying asterriquinones. Amino- and aziridinyl-asterriquinone were newly synthesized, but showed no activity. However, dimethylallylation of asterriquinone C1, which had no effect *in vivo*, yielded an active compound, with a change in pK_a value from 5.0 to 7.2. This suggests that other active compounds may be obtained by the chemical modification of asterriquinones with various other functional groups.

Keywords——asterriquinones; *Aspergillus terreus*; chemical modification; antitumor activity; Ehrlich carcinoma

In the previous papers, it was demonstrated that asterriquinone (ARQ) and some ARQ analogs (AQ A1—AQ D) isolated from *Aspergillus* fungi were effective in inhibiting the growth of several transplantable animal tumors *in vivo*,[2,3] and the action mechanism of these compounds might involve an effect on DNA function, due to binding to DNA molecules.[1] Furthermore, a correlation between the antitumor activity and pK_a value of these compounds was observed.[1] ARQ and its analogs were, however, found to exhibit unsatisfactory antitumor activities as compared with mitomycin C.

The present work was designed to obtain more potent compounds by the chemical modification of ARQ and related compounds.

Experimental[4]

Materials——ARQ[5] and ARQ analogs[6] were prepared by the methods reported previously from mycelia of *Aspergillus terreus* IFO 6123 and *Asp. terreus* var. *africanus* IFO 8835, respectively. Amino- and aziridinyl-ARQ were obtained by the reaction of dimethyl ether of ARQ (AQ A1) with ammonia and ethyleneimine in ethanol, respectively. Amino-ARQ, mp 275°C (EtOH), *Anal.* Calcd for $C_{32}H_{32}N_4O_2$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.32; H, 6.52; N, 10.80. Aziridinyl-ARQ, mp 258°C (EtOH), *Anal.* Calcd for $C_{36}H_{36}N_4O_2$: C, 77.67; H, 6.52; N, 10.06. Found: C, 77.97; H, 6.75; N, 10.16.

Bis-*N*,*N*′-(3,3-dimethylallyl)-AQ C1 was synthesized according to the method reported by Cardillo *et al.*[7] with a slight modification as follows; AQ C1 (470 mg) was dissolved in 10 ml of hexamethylphosphoric acid (HMPA) and added dropwise a suspension of NaH (120 mg, 2.5 eq mol) in 2 ml of HMPA under an N_2 atmosphere with ice-cooling. The whole was stirred for 8 h at room temperature, then 0.24 ml of dimethylallyl bromide (2.0 eq mol, Tokyo Kasei Kogyo) was added under ice-cooling, and the reaction mixture was stirred overnight at room temperature. Then, the mixture was extracted with 100 ml of C_6H_6 and the organic phase was washed with 10% HCl (twice, 100 ml each), 10% $NaHCO_3$ (twice, 100 ml each), and 10% NaCl (twice, 100 ml each), successively. The organic phase was concentrated *in vacuo*, and the residue was chromatographed on a silica gel column[8] (C_6H_6). The main purple fraction (450 mg, 95% yield) was collected. mp 62—65°C (MeOH), *Anal.* Calcd for $C_{39}H_{42}N_2O_4$: C, 77.71; H, 7.02; N, 4.65. Found: C, 78.02; H, 7.07; N, 4.51.

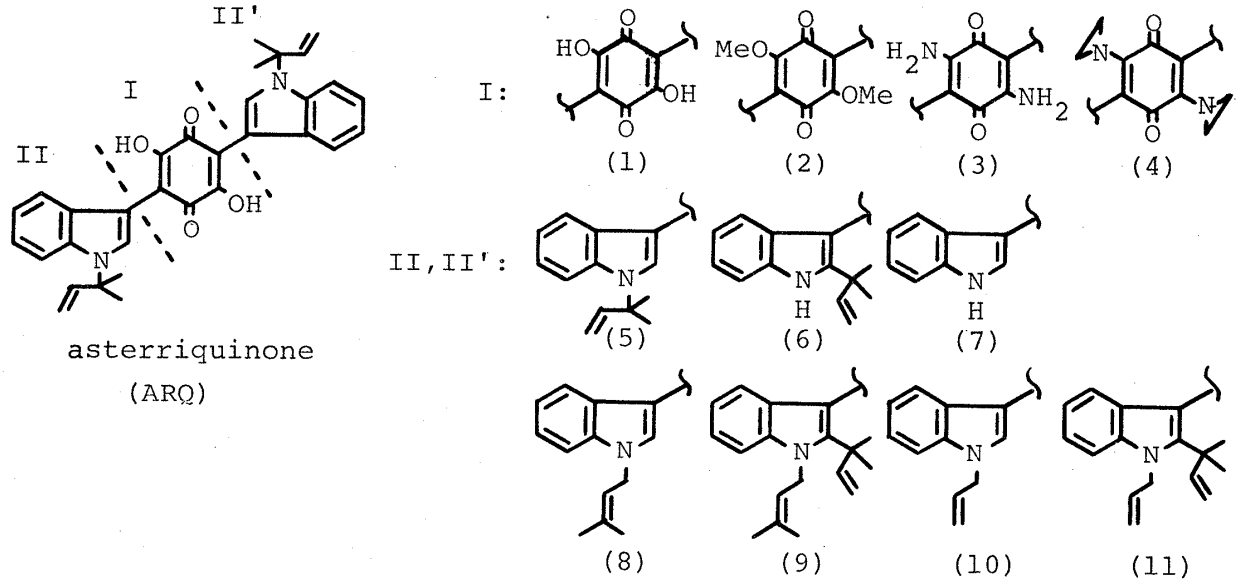
Bis-*N*,*N*′-(3,3-dimethylallyl)-demethyl-AQ C1 (AQ C1-2) was obtained by the demethylation of the above compound in alkaline solution[9] (80% yield). mp 89°C (MeOH), *Anal.* Calcd for $C_{37}H_{38}N_2O_4$: C, 77.32; H, 6.67; N, 4.87. Found: C, 77.17; H, 6.63; N, 4.52.

Bis-*N*,*N*′-(3,3-dimethylallyl)-demethyl-AQ D (AQ D-2) was synthesized from AQ D by the same procedure as in the case of AQ C1-2. mp 223—225°C (MeOH), *Anal.* Calcd for $C_{32}H_{30}N_2O_4$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.68; H, 5.77; N, 5.23.

N,*N*′-Diallyl-demethyl-AQ C1 (AQ C1-3) was also synthesized by the same procedure from AQ C1 using

allyl bromide instead of dimethylallyl bromide. mp 92—95°C (MeOH), *Anal.* Calcd for $C_{33}H_{30}N_2O_4$: C, 76.42; H, 5.83; N, 5.40. Found: C, 76.20; H, 5.83; N, 5.37.

The structures of all of the ARQ analogs thus obtained were assigned from the spectral data, *i.e.*, ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectra (MS) measurements, as shown in Fig. 1.



Compound	I	II, II′	Compound	I	II, II′	Compound	I	II, II′
Amino-ARQ	3	5, 5	AQ C1	2	6, 7	AQ D	2	7, 7
Aziridinyl-ARQ	4	5, 5	AQ C1-2	1	8, 9	AQ D-2	1	8, 8
			AQ C1-3	1	10, 11			

Fig. 1. Chemical Structures of the Modified Asterriquinone (ARQ) Derivatives

Animals——Male 5-week-old ddY mice were from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, and were kept on the breeding diet NMF (Oriental Yeast) with water *ad libitum* in an air-conditioned room during the experiments.

In Vivo Antitumor Experiments——Ehrlich carcinoma cells (2×10^6 or 4×10^6) were implanted intraperitoneally or subcutaneously (left inguinal region) into each mouse. The chemicals to be tested for antitumor activities were suspended in 0.25% carboxymethylcellulose (CMC) solution and administered intraperitoneally under the stated treatment schedule. In the experiments with the ascites tumor, the antitumor activity was evaluated at 60 d after the implantation by comparing the median survival time (MST) of the treated animals (T) with that of the control animals (C); namely, increase of life span (ILS) was assessed as $(T/C-1)\times100$ (%). In the experiments with the solid tumor, the antitumor activity was evaluated at 14 d after the implantation in terms of the ratio (T/C, %) by comparing the mean tumor mass weight in the treated animals (T) with that in the control (C).

Results and Discussion

The chemical structures of various ARQ analogs and chemically modified compounds are presented in Fig. 1.

Firstly, modification of the ARQ molecule was carried out by the replacement of functional groups at the 3 and 6 positions in the benzoquinone moiety. Tables I and II show that the modification of ARQ to the amino or aziridinyl derivative failed to enhance the antitumor potency. Benzoquinone derivatives having aziridinyl groups in the molecule, such as mitomycin C, carbazilquinone or E 39, are well known potent anticancer agents.[10] In spite of the introduction of an ethyleneimino moiety into the molecule, no increase in antitumor

Table I. Effect of the chemically Modified ARQ Derivatives on Ehrlich Carcinoma in Mice. 1. Ascites Tumor[a]

Compound[b]	Dose (mg/kg/d)	MST[c] (d)		ILS[d] (%)	60-d survivor
		Treated	Control		
ARQ	30	31.5	14.0	125	1/6
Amino-ARQ	30	17.0	16.0	6	0/6
Aziridinyl-ARQ	10	15.0	18.0	·	0/6
AQ C1-2	30	25.5	14.0	82	1/6
AQ C1-3	30	14.0	14.0	0	0/6
AQ D-2	30	17.0	14.0	21	0/6

a) Ehrlich ascites cells (2×10^6) were implanted intraperitoneally into ddY mice.
b) The compounds were administered intraperitoneally as a suspension in 0.25 % CMC solution for 7 successive days (days 1—7).
c) Median survival time.
d) Increase of life span.

Table II. Effect of the chemically Modified ARQ Derivatives on Ehrlich Carcinoma in Mice. 2. Solid Tumor[a]

Compound[b]	Dose (mg/kg/d)	Mean tumor weight ± S.E.[c] (g)		T/C [d] (%)
		Treated	Control	
ARQ	30	0.55±0.15	2.05±0.37	27
Amino-ARQ	30	4.95±1.27	4.97±0.76	100
Aziridinyl-ARQ	10	2.99±0.72	3.65±0.60	82
AQ C1-2	30	0.94±0.17	2.05±0.37	46
AQ C1-3	30	1.43±0.37	2.05±0.37	70
AQ D-2	30	1.36±0.23	2.05±0.37	66

a) Ehrlich ascites cells (4×10^6) were implanted subcutaneously to the left inguinal region of ddY mice.
b) The compounds were administered intraperitoneally as a suspension in 0.25% CMC solution for 10 successive days (days 3—12).
c) Standard error.
d) Ratio in tested/control.

activity was observed in the modified compound, possibly due to differences in the size and conformation of the molecules.

In other attempts to augment the antitumor potency, inactive AQ C1-1 was modified to dimethylallyl (AQ C1-2) and allyl derivative (AQ C1-3). The pK_a values for ionizing groups of these compounds were changed from 5.0 (AQ C1-1) to 7.2 (AQ Cl-2) and 6.6 (AQ C1-3), respectively. As shown in Tables I and II, AQ C1-2 was moderately effective on the ascites and solid tumors of Ehrlich carcinoma, while AQ C1-3 was not. AQ D-2, produced by dimethylallylation of AQ D, was also ineffective. These results suggest that, in order to obtain antitumor-active derivatives in the series of ARQ analogs, it may be neccessary not only to alter the pK_a value by alkylation, but also to introduce hydrophilic groups into the molecule.

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References and Notes

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