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ALTERPORRIOL A: A MODIFIED BIANTHRAQUINONE FROM *ALTERNARIA PORRI**

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Key Word Index—*Alternaria porri*; fungus; bianthraquinone; atropisomer; alterporriol A.

Abstract—Culture liquid and mycelia of *Alternaria porri* afforded a novel anthraquinone named alterporriol A, whose structure was determined by spectroscopic methods as well as by degradation to known compounds. Alterporriol A was found to be an atropisomer of alterporriol B previously reported.

INTRODUCTION

During the course of our investigation on the metabolic pigments of *Alternaria porri* (Ellis) Ciferri, the causal fungus of black spot disease of stone leek (Japanese name: negi), we reported that three dark red pigments were isolated from the culture liquid and mycelia, and were named alterporriol A (Ap-A, **1**), B (Ap-B, **2**) and C (Ap-C) in increasing order of R_f values on TLC [1]. Of these pigments the structure of **2** has already been determined [1]. This paper deals with the structural elucidation of another pigment Ap-A (**1**).

Repeated silica gel chromatography of the 5% sodium carbonate soluble part obtained from the culture liquid and mycelia, followed by purification using reversed phase HPLC, led to the isolation of **1**. Compound **1** represents a new type of bianthraquinone which was found to be an atropisomer of Ap-B (**2**) previously reported [1].

RESULTS AND DISCUSSION

The UV and IR spectra of compound **1** were found to be superimposable with those of **2**. The ^{1}H NMR spectral data of **1** and **2** resemble each other in many respects, as shown in Table 1. Comparing the ^{1}H NMR spectral data of **1** with those of macrosporin (**4**) and altersolanol A (**6**), both are metabolic pigments of *Alternaria porri* [2, 3], it seems that the ^{1}H NMR spectra of **4** and **6** adds up to that of **1**, although the β-hydroxyl proton signal in **4** is found in the deshielded region in **1** and **2** (**4**: δ9.70; **1**: 13.10, **2**: 13.06). Thus, compound **1** was presumed to be a modified bianthraquinone consisting of **4** and **6**, similar to compound **2**. This presumption was proved to be correct by the reductive cleavage of **1** with alkaline sodium dithionate, which afforded compounds **3**, **4**, **5** and **6**. Identifications were performed by comparing their R_fs with those of authentic samples by HPLC. It has been reported that 3,4,5-trihydroxy-7-methoxy-2-methylanthraquinone (**5**) [4] is susceptible to transformation to 2-methylxanthopurpurine-7-methyl ether (**3**) and **4** [5]. It seems that **4** and **6** are first formed on reductive cleavage and next **6** is transformed to **3** and **5**. Based on all the results obtained, it can be said that compound **1** is an isomer of **2**.

The attachment of the **6** unit to the **4** system follows from the ^{1}H NMR and ^{13}C NMR spectra of **1**. The ^{1}H NMR spectra of **4** and **6** shown in Table 1, exhibited the proton signals of C-6 H and C-8 H as a pair of doublets because of *meta*-coupling. In the spectral data of **1**, however, C-3 H (δ6.80) corresponding to C-6 H(4) (δ6.70) and C-3 H (δ6.78) corresponding to C-6 H(6) (δ6.71) are detected as singlets. These facts suggest that **1** is a modified bianthraquinone bonded at C-8 (**4**) C-8 (**6**) in a similar manner to **2**. Additional support was provided by the similarities of ^{1}H NMR (Table 1) as well as ^{13}C NMR data of **1** and **2** (Table 2). As shown in Table 1, additional evidence was provided by the ^{1}H NMR data which shared that the methoxyl groups of **1** (C-2 OMe, C-2 OMe) appeared in a considerable shielded region (δ3.63, 3.69; **4**: 3.91, **6**: 3.90) on account of the anisotropy caused by the aromatic half of the ring and that the down field shifts of the chelated hydroxyl protons at C-4, (δ13.10, 13.74; **4**: 12.86, **6**: 12.22) were in accord with the presence of the **6** moiety at the 8-position of **4** [4]. It may be possible to consider that the two halves of the molecule of **1** and **2** might be at *ca* right angles rotating to some extent about the position in solution.

It has been reported that some biphenyl-like compounds with *ortho*-substituted methoxyl groups exhibit atropisomerism with different degrees of stabilities. In these compounds, the steric size of the methoxyl groups seems to be large enough to obtain restricted rotation. For example, optically stable atropisomerism of knipholone (**7**) and primaquine dimer (**8**) have been reported as atropisomers due to the restricted rotation of their C–C linkages [6, 7]. Considering structure **1**, it could show atropisomerism, because the methoxyl groups are situated at the *ortho* positions of the C–C linkage connecting the monomeric halves. The ^{1}H NMR spectral data of Ap-A heptaacetate (**1a**) show that the chemical shifts of all the other acetyl protons except for C-8, (δ1.51; **2a**: 1.94) are in close agreement with those of **2a** and **6a** (Table 3). Thus, the remarkable high field shift of the C-8 alcoholic acetyl protons holds the key for the stereochemistry of **1a** and **2a**. In the two dimensional correlated spectroscopy

*Studies of the Metabolic Products of *Alternaria porri* Part 13. For Part 12 see ref. [1].

Table 1. ^{1}H NMR spectral data of altersolanol A, macrosporin, alterporriol A and B

As-A (6)	Mac (4)	Ap-A (1)	Ap-B (2)
δ(ppm)			
4.41 (C-1 H) *s*		4.26 (C-8′ H)	4.29 (C-8′ H) *s*
3.77 (C-3 H) *d* J_{3-4} = 6.6 Hz		3.69 (C-6′ H) *d* $J_{5'-6'}$ = 6.5 Hz	3.76 (C-6′ H) *d* $J_{5'-6'}$ = 6.5
4.64 (C-4 H) *d* J_{3-4} = 6.6 Hz		4.63 (C-5′ H) *d* $J_{5'-6'}$ = 6.5 Hz	4.70 (C-5′ H) *d* $J_{5'-6'}$ = 6.5
6.71 (C-6 H) *d* J_{6-8} = 2.2 Hz		6.78 (C-3′ H) *s*	6.77 (C-3′ H) *s*
7.11 (C-8 H) *d* J_{6-8} = 2.2 Hz		— (C-1′ H)	— (C-1′ H)
1.37 (C-2 Me)		1.27 (C-7′ Me) *s*	1.26 (C-7′ Me) *s*
3.90 (C-7 OMe)		3.69 (C-2′ OMe) *s*	3.67 (C-2′ OMe) *s*
	7.99 (C-1 H)	7.70 (C-8 H) *s*	7.69 (C-8 H) *s*
	7.51 (C-4 H)	7.50 (C-5 H) *s*	7.50 (C-5 H) *s*
	6.70 (C-6 H) J_{6-8} = 2.2 Hz	6.80 (C-3 H) *s*	6.77 (C-3 H) *s*
	7.27 (C-8 H) J_{6-8} = 2.2 Hz	— (C-1 H)	— (C-1 H)
	2.32 (C-2 Me) *s*	2.23 (C-7 Me) *s*	2.54 (C-7 Me) *s*
	3.91 (C-7 OMe) *s*	3.63 (C-2 OMe) *s*	3.68 (C-2 OMe) *s*
	9.70 (C-3 OH) *s*	13.10 (C-6 OH) *s*	13.06 (C-6 OH) *s*
12.22 (C_5-OH) *s*	12.86 (C-5 OH) *s*	13.74 (C-4 and C-4′ OH) 2H, *s*	13.70 (C-4 and C-4′ OH) 2H *s*

As-A: altersolanol A; Mac: macrosporin; Ap-A: alterporriol A; Ap-B: alterporriol B.
In d_8-THF with TMS (400 MHz).

Table 2. ^{13}C NMR spectral data of alterporriols A and B (INEPT method)

		Ap-A	Ap-B		Ap-A	Ap-B
Me:	C-7	16.53	16.76	C-4′a	111.61	111.65
	C-7′	22.35	23.46	C-9a	127.40	126.71
OMe:	C-2	56.96	56.63	C-7	131.40	130.93
	C-2′	56.96	56.63	C-10a	132.70	133.76
–CH:	C-8′	70.13	69.93	C-1a	133.52	133.18
	C-5′	70.71	70.74	C-1′a	134.46	134.62
	C-6′	75.22	75.46	C-9′a	144.10	144.01
	C-3′	103.90	104.41	C-10′a	145.30	145.05
	C-3	104.31	104.50	C-6	162.70	162.71
	C-5	111.53	110.97	C-4′	165.40	165.05
	C-8	131.11	130.79	C-4	165.40	165.27
ter-C:	C-7′	76.61	74.43	C-2′	166.00	165.41
	C-4a	105.10	104.32	C-2	166.70	166.26
	C-1′	109.30	111.20	C-9′	182.00	182.40
	C-1	111.08	111.29	C-9	185.70	186.17
				C-10	187.90	188.20
				C-10′	190.70	190.67

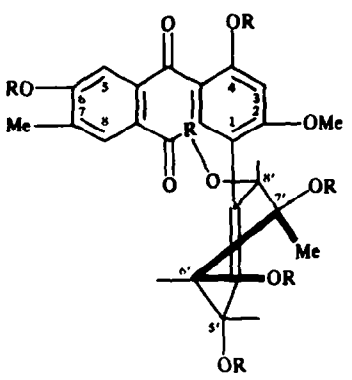
Ap-A; alterporriol A; Ap-B; alterporriol B.
In CD_3OD.

(COSY) and nuclear overhauser effect (NOESY) of **2a**, a cross peak between δ1.94 (C-8′, alcoholic acetyl protons) and δ3.71 (C-2 methoxyl protons) was observed. However, the cross peak corresponding to the relation was not observed in the COSY of **1a**. The remarkable high field shift of the C-8′ acetyl protons in **1a** might be due to the anisotropy caused by the aromatic ring of macrosporin (**4**). From these spectral data, the spatial structures of **1** and **2** can be depicted as in Fig. 1 as one of the most possible conformations.

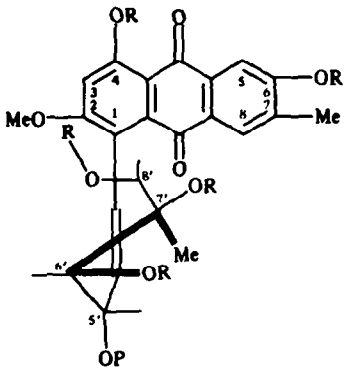
EXPERIMENTAL

Extraction and isolation of alterporriol A. The cultural conditions using the stone-leek decoction as a medium and the method of isolation have already been reported [1]. Crude Ap-A obtained by prep. TLC was further purified by reversed phase HPLC (column: YMA-312 ODS-type) with a solvent system of MeCN–H_2O (3:7). Yields of Ap-A were 245 mg from 138 l culture medium.

Alterporriol A. Dark-red amorphous, mp 300° (dec.); $[\alpha]_D^{25}$ −235° (EtOH; *c* 0.05). IR ν_{max}^{KBr} cm^{-1}: 3700–3500 (OH), 1670,

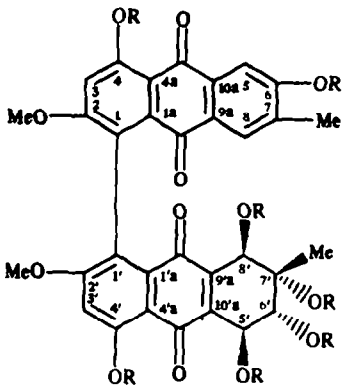


	R
1	H
1a	Ac

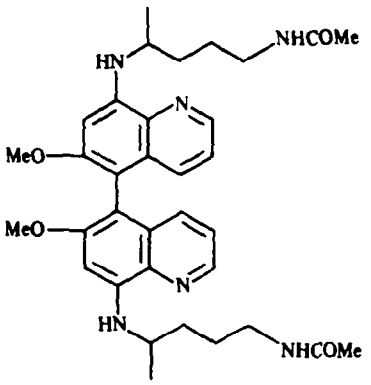


	R
2	H
2a	Ac

Fig. 1.



	R
1	H
1a	Ac
2	H
2a	Ac



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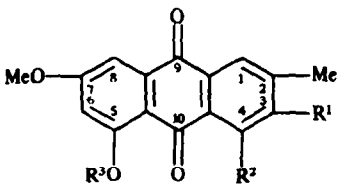

	R^1	R^2	R^3
3	H	H	H
4	OH	H	H
5	OH	OH	OH

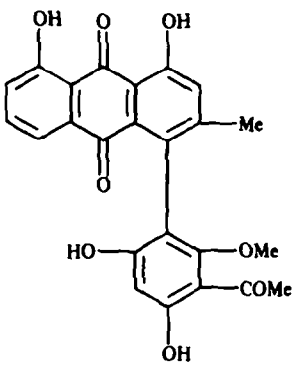

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Table 3. Assignments of alcoholic acetyl protons

	Ap-A heptaacetate (1a)	Ap-B heptaacetate (2a)	As-A* pentaacetate (6a)
Carbon number attached to *O*-acetyl group	δ(ppm)		
8′ (1)	1.51	1.94	1.98
5′ (4)	1.95	1.96	2.06
6′ (3)	2.09	2.09	2.13
7′ (2)	2.14	2.12	2.15

( ): numbering as in altersolanol A (As-A).
In $CDCl_3$ with TMS (400 MHz).
*Values documented in ref. [5].

1650 (free C=O), 1640 (chelated C=O), 1210 (Ar-OH), 1160 (tertiary OH), 1110 (sec. OH); UV λ_{max}^{EtOH} nm (log. ε) 228 (4.45), 280 (4.38), 310 (4.09), 4.05 (3.79), 435 (3.82). MS(FD) *m/z*: 618 $[M]^+$ ($C_{32}H_{26}O_{13}$, 23), 582 ($C_{32}H_{22}O_{11}$, $[M-2H_2O]^+$, 100), 566 ($C_{32}H_{22}O_{10}$, $582+2H-H_2O$), MS (SIMS) *m/z* 641 $[M+Na]^+$. According to the M_r deduced from MS, Ap-A has the molecular formula $C_{32}H_{26}O_{13}$ (618). The ^1H NMR data (400 MHz, d_8-THF) are shown in Table 1.

Alterporriol A heptaacetate (1a). On acetylation with Ac_2O containing a few drops of 70% perchloric acid, followed by purification using reversed phase HPLC (column: ODS-type, YMC-A343) with a solvent system of MeCN–H_2O (11:9). Ap-A (20 mg) gave a yellow acetate (14 mg), mp 218°. Elemental analysis of 1a gave C, 59.99 H, 4.33 for the suggested empirical formula $C_{46}H_{40}O_{20}$ (calc. C, 60.52, H, 4.42). The FDMS gave a $[M]^+$ at *m/z* 912, corresponding to $C_{32}H_{19}O_{13}$ $(COCH_3)_7$ (912.78) and two groups of peaks. These are 912–870–828–786 and 734–692–650–608–566, spaced by 42 mass units corresponding to a difference of CH_2CO, which supports a heptaacetate structure. IR ν_{max}^{KBr} cm^{-1}: 3450, 2920, 1750, 1670, 1570, 1460, 1380, 1340, 1290, 1200; ^1H NMR $\delta_{Me_4Si}^{CDCl_3}$ 1.47 (3H, *s*, C-7 Me), 1.51, 1.95, 2.09, 2.11, (3H, each, *s* ROCOMe), 2.23 (3H, *s*, C-7 Me), 2.34, 2.44, 2.52 (3H, each *s*, Ar-OCOMe), 3.74, 3.75 (3H, each *s*, Ar-OMe), 5.44 (1H, *d*, *J* = 7.3 Hz, C-6 H), 6.25 (1H, *d*, *J* = 7.3 Hz, C-5 H), 6.67 (1H, *s*, C-8 H), 6.89 (1H, *s*, C-3 H), 6.93 (1H, *s*, C-3 H), 7.80 (1H, *s*, C-5 H), 7.84 (1H, *s*, C-8 H). In accordance with these assignments, Ap-A can be converted into its heptaacetyl derivative (1-a).

Reductive cleavage of 1. To a soln of 1 (2 mg) in N NaOH (5 ml), an aq. soln of $Na_2S_2O_4$ (5 mg) in 5 ml H_2O was added. After heating at 70° for 30 min, the reaction mixt. was cautiously neutralized under cooling and extd with EtOAc. After evapn of the dried extract (Na_2SO_4), the residue obtained was analysed by HPLC. For analysis, a YMC-A314 ODS-type column was used with a mobile phase of MeOH–H_2O (7:3) for 30 min, which was then increased to 100% MeOH during the next 40 min. Compounds 4, 5 and 6 were identified by comparing their R_ts with those of authentic samples [3–5].

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