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

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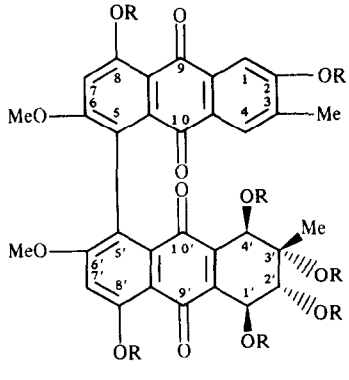
Key Word Index—*Alternaria porri*; α,β′-bianthraquinone; alterporriol C.

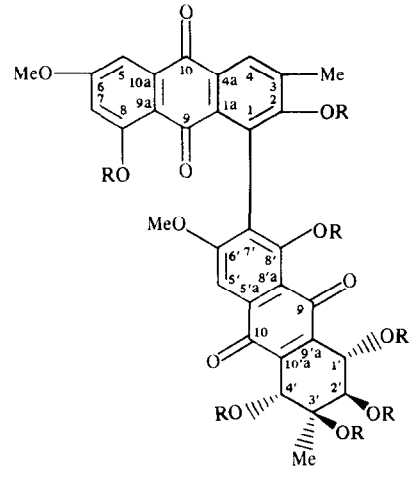
Abstract—The spent growth medium and mycelia of *Alternaria porri* afforded a novel anthraquinone named alterporriol C, whose structure was determined by spectroscopic methods as well as by degradation to known compounds. Alterporriol C was found to be an α,β′-bianthraquinone derived from macrosporin and altersolanol A.

INTRODUCTION

In the course of our investigation on the pigments of *Alternaria porri* (Ellis) Ciferri, the causal fungus of black spot disease of stone-leek (Japanese name: negi), we isolated three dark red pigments from the culture liquid and mycelia [1]. The structures of two of these pigments, alterporriol A (Ap-A, **1**) and B (Ap-B, **1**), have already been determined and found to be atropisomers of each other [1, 2]. This paper deals with the structural elucidation of the third pigment (Ap-C, **2**). Cladofulvin [3] and asphodelin, [4] have been reported as naturally occurring α,β′-bianthraquinone. The former is a product of *Cladosporium fulvum* and the latter is a component of *Aloe saponaria* HAW. Ap-C is the second example of an α,β′-bianthraquinone of fungal origin.



	R
1	H
1a	Ac



	R
2	H
2a	Ac

RESULTS AND DISCUSSION

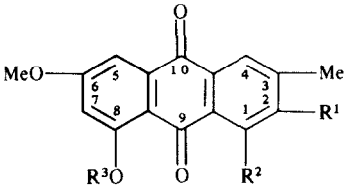
When the ^{1}H NMR spectrum of Ap-C was compared with those of macrosporin (**3**) and altersolanol A (**4**), both are metabolic pigments of *Alternaria porri* [5, 6], it was obvious that the spectrum of Ap-C was the sum of those of **3** and **4** (Table 1). This suggested Ap-C was a modified bianthraquinone formed from **3** and **4**. To confirm the identities of the structural fragments of Ap-C, the reductive cleavage products formed on treatment of Ap-C with alkaline sodium dithionate were examined. Compounds **3**, **4** and 1,2,8-trihydroxy-6-methoxy-3-methylanthraquinone (**5**) were identified by comparing their R_fs with those of authentic samples [1]. The combined results showed that Ap-C is another modified bianthraquinone derived from the monomeric units **3** and **4**.

The manner of the attachment of **4** to **3** followed from the ^{1}H NMR spectrum of Ap-C. As shown in Table 1, the

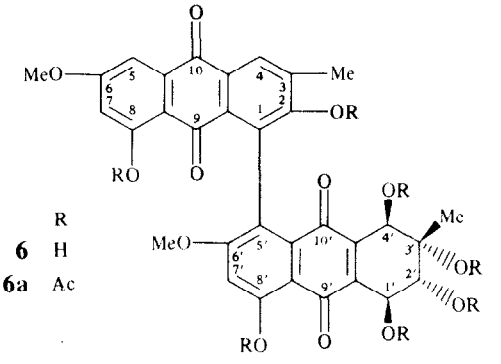
*Studies of the Metabolic Products of *Alternaria porri*, part 14. For Part 13 see ref. [2].

Table 1. ^{1}H NMR spectral data of macrosporin (**3**), altersolanol A (**4**) and alterporriol C (**2**) (400 MHz, d_8-THF with TMS)

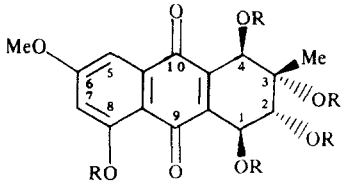
H	3	H	4	H	2
4	8.10 *s*			4	8.10 *s*
1	7.51 *s*			1	
7	6.70 *d* J = 2.2 Hz			7	6.61 *d* J = 2.2 Hz
5	7.27 *d* J = 2.2 Hz			5	7.25 *d* J = 2.2 Hz
Me-3	2.32 *s*			Me-3	2.32 *s*
OMe-6	3.91 *s*			OMe-6	3.88 *s*
OH-2	9.70 *s*	R-OH × 4	2.58 *br, s*	OH-5	12.59
OH-8	12.86 *s*	OH-8	12.21 *s*	and 8′	13.08
		4	4.41 *s*	4′	4.30 *s*
		2	3.77 *d* J = 6.6 Hz	2′	4.15 *d* J = 6.5 Hz
		1	4.64 *d* J = 6.6 Hz	1′	4.96 *d* J = 6.5 Hz
		7	6.71 *d* J = 2.2 Hz	7′	
		5	7.11 *d* J = 2.2 Hz	5′	6.85 *s*
		3	1.37 *s*	Me-3′	1.27 *s*
		OMe-6	3.90 *s*	OMe-6′	3.71 *s*



	R^1	R^2	R^3
3	OH	H	H
3a	OAc	H	Ac
5	OH	OH	H



	R
6	H
6a	Ac



	R
4	H
4a	Ac

^{1}H NMR spectra of **3** and **4** exhibit the signals of H-5 and H-7 as a pair of doublets because of *meta*-coupling. In the spectrum of Ap-C, a pair of doublets appear at δ 6.61 and 7.25 corresponding to H-7 (δ 6.70) and H-5 (δ 7.27) in **3**, respectively. In addition, an aromatic proton signal at δ 8.10 in Ap-C can be attributed to H-4 (δ 8.10) in **3**. Moreover, the 2D ^{1}H NMR (NOESY) spectrum of Ap-C heptaacetate (**2a**) shows that the signal due to the aromatic proton at δ 8.21 (H-4) is correlated to the aromatic methyl protons at δ 2.26 (Me-3). These results suggest that the **3** moiety of Ap-C is linked at C-1 to the **4** moiety, and it follows that the internuclear C–C link must be C-1 to C-7′ (**2**) or C-5′ (**6**).

Ogihara *et al.* have reported [7] that in the ^{1}H NMR spectra of acetoxylated bianthraquinones the signals due to the protons of acetate groups *ortho* to the internuclear bond resonate at higher field than usual on account of anisotropy caused by the aromatic ring of the other 'half' of the molecule. In the ^{1}H NMR data of Ap-A previously reported [2], the signals due to methoxyl protons *ortho* to the internuclear C–C link resonate at higher field than those of its constituents, **3** and **4**. In the case of **2a**, a methoxyl group (OMe-6′) and two acetoxyl groups (OAc-2 and OAc-8′) resonate at unusually high field as shown in Table 2. Namely, signals of OMe-6′(δ 3.70; 4a: 3.96), OAc-2(δ 2.14; 3a: 2.33) and OAc-8′(δ 2.10; 4a: 2.38) are observed. Hence, the structure of **2** and **2a** are compatible with those of Ap-C and Ap-C heptaacetate. The structure **6** must be excluded, because it has a methoxyl and an acetoxyl group located *ortho* to the internuclear C–C linkage.

EXPERIMENTAL

Extraction and isolation of alterporriol C. The cultural condition using the stone-leek decoction as a medium and the method of isolation have already been reported [1]. Crude Ap-C obtained by prep. TLC was purified by reversed phase HPLC: (YMC-S343 ODS-type, solvent MeCN–H_2O 3:7). Yields of Ap-C were 81 mg from 30 l culture medium.

Table 2. Assignment of acetyl and methoxyl protons (400 MHz, $CDCl_3$ with TMS)

Ap-C heptaacetate (**6a**)		Macrosporin diacetate (**3a**)		As-A pentaacetate (**4a**)*	
1.91	(C-4′)†			1.98	(C-4)
1.94	(C-1′)			2.06	(C-1)
2.02	(C-2′)			2.13	(C-2)
2.08	(C-3′)			2.15	(C-3)
2.10	(C-8′)			2.38	(C-8)
2.14	(C-2)	2.33	(C-2)		
2.27	(C-8)	2.31	(C-8)		
3.70	(C-6′)			3.96	(C-6)
3.96	(C-6)	3.93	(C-6)		

*Values documented in ref. [8].
†Carbon number attached to OAc and OMe group.

Table 3. ^{13}C NMR spectral data of alterporriol C heptaacetate (**2a**) (INEPT method) (400 MHz, $CDCl_3$ with TMS)

Me	C-3	16.83	*quat.* -C	C-3′	80.54	OCOMe		166.64
	C-3′	17.01		C-9′a	116.74			167.16
OAc		19.67		C-7′	116.74			168.04
		20.36		C-9a	118.57			168.17
		20.57		C-1	125.13			168.41
		20.59		C-4a	129.81			168.81
		20.92		C-1a	130.74			169.67
		21.08		C-10′a	132.40	C=O	C-9	179.67
		21.85		C-10a	135.69		C-9′	180.16
OMe	C-6	55.89		C-3	136.13		C-10	180.91*
	C-6′	56.53		C-5′a	137.97		C-10′	181.27†
–CH	C-4′	64.00		C-8′a	141.87			
	C-1′	66.89		C-8′	150.05			
	C-2′	72.85		C-8	150.77			
	C-5	108.65		C-2	151.30			
	C-5′	109.60		C-6	161.39			
	C-7	115.30		C-6′	163.37			
	C-4	129.15						

Assignments of * and † may be reversed, respectively.

Alterporriol C. Dark-red amorphous, mp > 300° (dec.), $[\alpha]_D^{25}$ −268° (EtOH; *c* 0.05). IR ν_{max}^{KBr} cm^{-1}: 3600–3000 (OH), 1670, 1650 (free C=O), 1640 (chelated C=O), 1210 (Ar–OH), 1100 (*sec* OH); UV λ_{max}^{EtOH} nm (log. ε) 228 (5.91), 267 (5.68), 320 (5.33), 360 (4.51), 422 (4.69), 450 (4.69); MS(FD) (rel. int.) *m/z*: 618 $[M]^+$(24) 582 $[M - 2H_2O]^+$ (100), 566; MS(SIMS) *m/z* (rel. int.): 643 $[M + Na + 2H]^+$ (4), 621 $[M + 3H]^+$ (44), 567.1306 (100), Calc. for $C_{32}H_{23}O_{16}$, *m/z* 567.1285.

Alterporriol C heptaacetate (**2a**). On acetylation with Ac_2O containing a few drops of 70% perchloric acid, followed by purification using HPLC (YMC-S343 ODS-type, solvent MeCN–H_2O 11:9), Ap-C (20 mg) gave a yellow acetate (8 mg), mp 270–271°. MS(FD) *m/z*: 912. $[M]^+$, corresponding to $C_{32}H_{19}O_{13}Ac_7$ (912.78); NMR $\delta_{Me_4Si}^{CDCl_3}$: 1.53 (3H, *s*, Me-3′), 2.41 (3H, *s*, Me-3), 1.91, 1.94, 2.02, 2.08, 2.10, 2.14, 2.27 (3H, each, *s*, ROCOMe), 3.70, 3.96, (3H, each, *s*, Ar-OMe), 5.53 (1H, *d*, *J* = 7.3 Hz, H-2′), 6.21 (1H, *d*, *J* = 7.3 Hz, H-1′), 6.84 (1H, *s*, H-4′), 6.79 (1H, *s*, H-5′), 6.80 (1H, *d*, *J* = 2.6 Hz, H-7), 7.70 (1H, *d*, *J* = 2.6 Hz, H-5) 8.20 (1H, *s*, H-4); ^{13}C NMR: Table 3. The assignments were based on comparison with the chemical shifts of **3a** and **4a** [8].

Reductive cleavage of Ap-C. To a sol of Ap-C (2 mg) in 0.5 M NaOH (5 ml), an aq. sol of $Na_2S_2O_4$ (5 mg) in 5 ml H_2O was added. After heating at 70° for 30 min, the reaction mixture was cautiously neutralized with cooling and extd with EtOAc. After evapn of the dried extract (Na_2SO_4), the residue 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 **3**, **4** and **5** were identified by comparing their R_fs with those of authentic samples.

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