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BIOSYNTHESIS OF MACROSPORIN BY *ALTERNARIA PORRI**

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Key Word Index—*Alternaria porri*; biosynthesis of macrosporin

Abstract—The biosynthesis of macrosporin, a metabolite of *Alternaria porri*, was elucidated by incorporation experiments of single and double labelled acetates. ^{13}C NMR assignments were also established.

INTRODUCTION

Macrosporin (**1**) [1] is of interest as a metabolite of the important phytopathogenic fungi, *Alternaria porri* (Ellis) Ciferri, *Alternaria solani* and *Alternaria bataticola*, whose hosts are stone-leek, potato and sweet potato, respectively [2–4].

Fungi are known to form anthraquinones by linear combination of acetates, namely, octaketide chains [5]. Concerning the biosynthesis of **1**, Stoessl *et al.* have shown, by NMR [6] that cultures of fungus *Alternaria solani* incorporate [1, 2-$^{13}C_2$] and [1-^{13}C, 2-$^{2}H_3$] acetate into **1** from an octaketide chain. However, in their interpretation on the formation of anthraquinone skeleton from octaketide via condensation between methylene and keto groups some problems remain to be solved concerning the condensation pattern (Scheme 1). Namely, it is not from the small values (0.03–0.07 ppm) to discuss the isotopic shifts and assignments of the carbons.

We have already isolated interesting modified bianthraquinone derivatives, alterporriol A (Ap-A), B (Ap-B) and C (Ap-C), consisting of **1** and altersolanol A **2**, as metabolic pigments of *Alternaria porri* and their chemical structures have been determined [7–9]. The elucidation of their biosynthesis is another interesting problem. This prompted us to investigate the biosynthesis of **1**, a half moiety of Ap-A, B and C.

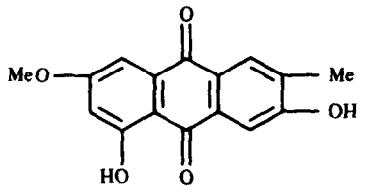
We report a spectroscopic study on the biosynthesis of **1** utilizing single and double labelled acetates, i.e. sodium [1, 2-$^{13}C_2$], [1-^{13}C] and [2-^{13}C] acetate to confirm the validity of octaketide pathways.

RESULTS AND DISCUSSION

In order to assign the chemical shifts of ^{13}C in **1**, first the ^{1}H-^{13}C 2D NMR (COSY) spectrum of **1** was examined before the biosynthetic elucidation. The ^{1}H NMR spectrum of **1** in d_8-THF exhibits the proton signals of C-7 H(δ6.70), C-5 H(δ7.26), C-1 H(δ7.52) and C-4 H(δ7.98) [8]. The ^{1}H–^{13}C 2D NMR (COSY) spectrum shows that the proton signals of C-7 H, C-5 H, C-1 H and C-4 H are correlated to the carbon signals of 106.1, 108.0, 111.6 and 131.1 ppm, respectively. Hence, assignments of C-7, -5, -1 and -4 were determined.

As shown in Table 1, chemical shifts of δ16.4 and 56.4 are apparently attributable to 3-Me and 6-OMe, respectively. And δ162.3, 166.4 and 167.4 are in each case attributable to one of the aromatic carbons bonded to oxygens, i.e. C-2, -8 and -6.

When double labelled acetates were administered, sharp satellite peaks were observed and ^{13}C–^{13}C coupling constants of **1** were given in Table 1. The ^{13}C–^{13}C coupling was observed between C-5 and C-6 (J = 65.9 Hz) and C-7 and C-8 (J = 69.9 Hz). Then, the peaks δ167.4, 166.4 and 162.3 can be attributable to C-6, C-8 and C-2, respectively. Other peaks remaining at δ133.0, 134.6, 126.9, 136.5 and 111.5 can be attributable to aryl carbons, i.e. one of C-3, -1a, -4a, -5a and -8a, of which the peak at δ133.0 can be attributable to C-3, because ^{13}C–^{13}C coupling was observed between Me and C-3 (J = 44.3 Hz). The peak at δ111.5, which appeared at a higher magnetic



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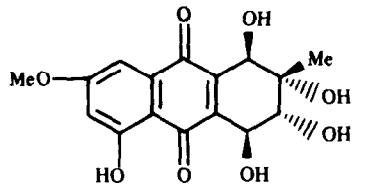

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*Part 15 of 'Studies on the Metabolic Products of *Alternaria porri*', for Part 14 see ref. [9]

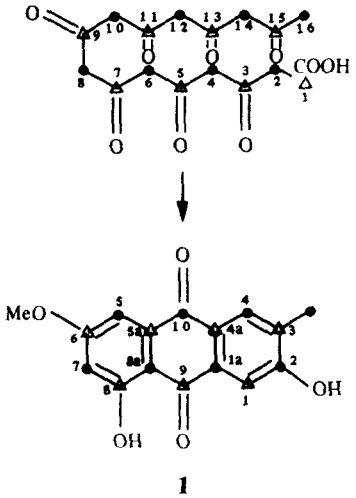
Table 1 Incorporation of sodium [2-^{13}C], 1-^{13}C] and [1,2-$^{13}C_2$] acetate into macrosporin

C	Chem shift (ppm)	J (Hz)	Natural (%)	[2-^{13}C]Acetate Enrichment		[1-^{13}C]Acetate Enrichment	
1	111 6	63 2	97 2	102 8%		225 8%	△
1a	134 6	63 2	38 5	115 5	●	44.0	
2	162 3	—	45 1	140 7	●	52 4	
3	133.0	44 3	54 4	51 9		121 9	△
4	131 1	59 2	94 9	252 5	●	89 8	
4a	126 9	59.2	38 6	42 2		95 2	△
5	108 0	65 9	88 3	256.3	●	87 9	
5a	136 5	52 4	42 7	36 9		93 4	△
6	167 4	65.9	48 9	46 3		99 6	△
7	106 1	69 9	90.6	253 5	●	80 7	
8	166 4	69 9	57 8	65 1		119 9	△
8a	111 5	57 8	31 9	95 3	●	42 9	
9	187 9	57.8	35.6	35 1		67 2	△
10	181 2	52 4	32 0	86 3	●	26 4	
3-Me	16 4	44 3	82 7	211 5	●	72 3	
6-OMe*	56 4	--	100 0	100 0		100 0	

In d_8-THF with TMS.
*Intensity of signal for OMe was defined as 100%
—no satellite peak
● enriched with [2-^{13}C]acetate
△ enriched with [1-^{13}C]acetate

field than those of other angular carbons, can be attributable to C-8a, because C-8a is adjacent to the carbon bearing a hydroxyl group [10]. Then, the peak at δ111.5 can be attributable to C-8a. As the ^{13}C–^{13}C coupling was observed between C-8a and C-9 (J = 57 8 Hz), the peak at δ187.9 can be attributed to C-9 and another carbonyl peak at δ181.2 must be due to C-10. The remaining aromatic carbons, i.e C-1a, -4a and -5a can be attributed to δ134 6, 126 9 and 136.5, respectively, because ^{13}C–^{13}C couplings were observed between C-1a and -1 (J = 63 2 Hz), C-4a and -4 (J = 59 2 Hz) and C-5a and -10 (J = 52.4 Hz).

Furthermore, to establish the condensation pattern of eight acetates, the single labelled acetates, sodium [2-^{13}C] and [1-^{13}C] acetate, were used. In each case, the signals for carbons incorporating ^{13}C in **1** were significantly enhanced relative to those for unlabelled naturally occurring **1** (Table 1).



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Scheme 1

Thus, as expected, **1** was proved to be derived from octaketide, in which eight acetate units were condensed in head to tail type, followed by the loss of carbonyl carbon from the terminal unit as shown in Scheme 1

EXPERIMENTAL

Reagents and apparatus Na [2-^{13}C], [1-^{13}C] and [1, 2-$^{13}C_2$] acetate (99 atom % ^{13}C) was purchased from Aldrich

Incorporation of Na [2-^{13}C], [1-^{13}C] *and* [1, 2-$^{13}C_2$] *acetate into macrosporin* For the biosynthetic studies, onion decoction (100 ml) including labelled acetate (15 mg) and unlabelled acetate (34 5 mg) was used as a medium After fermentation for 3 weeks at 25°, **1** was obtained according to the isolation procedure previously reported [1] and subjected to ^{13}C NMR analysis

REFERENCES

1 Suemitsu, R, Nakajima, M and Hiura, M (1961) *Agric. Biol Chem* **25**, 100
2 Suemitsu, R, Matsui, Y and Hiura, M (1957) *Bull Agric Chem Soc Jpn* **21**, 1
3 Stoessl, A (1969) *Can J Chem* **47**, 767.
4 Nakajima, S (1973) *Pharm Bull* **21**, 2083
5 Turner, W G and Aldrige, D C (1983) *Fungal Metabolites II*, p 140 Academic Press, London
6. Stoessl, A, Unwin, C H and Stothers, J B (1983) *Can J Chem* **61**, 372
7 Suemitsu, R, Yamamoto, T, Miyai, T and Ueshima, T (1987) *Phytochemistry* **26**, 3221
8 Suemitsu, R., Sano, T., Yamamoto, M, Arimoto, Y, Morimatsu, F and Nabeshima, T (1984) *Agric Biol Chem* **48**, 2611
9 Suemitsu, R, Ueshima, T, Yamamoto, T and Yanagawase, S (1988) *Phytochemistry* **27**, 3251
10 Berger, Y and Castonguay, A (1978) *Org Magn Reson* **11**, 375