Tetrahedron Letters No. 27, 2481 - 2484.

0040-4039/79/0701-2481$02.00/0

METABOLITES OF ALTERNARIA SOLANI PART V.[a] BIOSYNTHESIS OF ALTERSOLANOL A AND INCORPORATION OF ALTERSOLANOL A-$^{13}C_x$ INTO ALTERSOLANOL B AND MACROSPORIN

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The biosynthesis of the tetrahydroanthraquinone, altersolanol A (1), by Alternaria solani has been established by the incorporation of [1,2-$^{13}C_2$]-acetate. A blocked culture of A. solani was utilized to reveal that 1 is metabolized to altersolanol B and macrosporin.

Altersolanol A (1) and B (2), members of a very small group of naturally occurring tetrahydroanthraquinones, are of interest as antibiotics[5] and as metabolites of the important plant-pathogenic fungi, Alternaria solani[1-4] and A. porri.[6] These compounds have also been isolated from Phomopsis juniperivora[7] and Dactylaria lutea,[5] while 2 has recently been synthesized[8] as a simpler analog of the anthracyclinones. We now report that 1 is metabolized by A. solani to 2 and macrosporin (4) and possibly to 3, the newly discovered altersolanol C.

The A. solani strain (ATCC 11078) used in our earlier work[1-4] has become partially blocked, producing no more than trace amounts of pigments. Initial biosynthetic studies, using ^{13}Cmr methodology, were therefore carried out with a strain of A. solani kindly provided by Dr. U. Matern, Albert Ludwigs Universität, Freiburg. A representative experiment furnished 1 (48 mg from two 100 mL portions of still culture on medium T[2], supplemented with sodium acetate-1,2-$^{13}C_2$ (90% enriched) 7 days after inoculation) during 9 days' growth. The incorporation of intact acetate units followed the expected pattern (heavy bonds in 1) giving 0.7% enriched 1-$^{13}C_x$, (see Table) with a discernible starter effect revealed by the C_2-CH_3 unit (1.0% ^{13}C incorporation). In addition, zinniol (6) (14 mg, 1.5% ^{13}C enrichment), identified by direct comparison of its diacetate with an authentic specimen from A. zinniae,[9] and a small amount of 3 were isolated. The structure of 3 followed from its spectroscopic properties, determined with the aid of additional material from subsequent experiments: uv, λ_{max} 420, 284 (sh), 269, 238, 220 nm, log ε, 3.74, 3.99, 4.19, 3.97, 4.61; ^{1}Hmr (DMSO-d_6) δ: 1.28 (s, 2-Me), 2.79 (d of d, 4β-H), 2.35

[a] Parts I to IV: references 1-4, respectively.

(d of d, 4α-H) $J_{4\alpha4\beta}$ ~19 Hz, 3.77 (m, 3-H), 3.90 (s, 7-OMe), 5.52 (s, 1-H), 6.80 (d, J~2.5 Hz, 6-H), 7.03 (d, J~2.5 Hz, 8-H); ^{13}Cmr (see Table). Acetylation ($Ac_2O/HClO_4$/0°) gave a tetra-acetate: ^{1}Hmr ($CDCl_3$) δ: 1.96 (2-OAc), 2.08 (3-OAc), 2.13 (1-OAc), 2.43 (5-OAc) and 7.00 (s, 1-H). These signals were readily assigned by comparison with the published data[2,3] for 1 and 2.

An ethanolic solution (20 mL) of 1-$^{13}C_x$ (22 mg) from the main experiment was added in equal portions to four 100 mL cultures (medium T) of vigorously growing strain ATCC 11078 and the culture filtrates and mycelia were harvested after 9 days. After extraction (EtOH) and chromatography, the mycelia furnished 4 (5 mg, identified by tlc, uv, mp and mixture mp) which was converted to the diacetate. The ^{13}Cmr spectrum (see Table) of the diacetate (^{13}C enrichment: 0.7% at C_2-CH_3, 0.5% elsewhere, pattern as shown in 4) showed that approximately 70% of the sample had been derived from 1-$^{13}C_x$. The lower enrichment level observed is consistent with the production of ca. 1.5 mg of 4 found for a concurrent control experiment.

The filtrate from the 1-$^{13}C_x$ supplemented culture on extraction (EtOAc) and chromatography, yielded 4 (0.6 mg by uv), 2 (1 mg) and a trace of 3, identified by tlc; 1 was present in barely detectable traces. Dehydration[2] of 2-$^{13}C_x$ gave 5, identified by tlc and shown to be significantly enriched in ^{13}C by comparison of its mass spectrum with that of authentic material. The filtrate from the control cultures contained traces of 2 and 4 but no detectable quantities of 1 and 3.

These results establish that 1 is rapidly metabolized by A. solani to 2 and 4, possibly on branching pathways. It seems likely that 3 is also formed from 1 by enzymic agency and may give rise to 4 by a dehydration which need not be enzyme catalyzed. The results are also consistent with the concept that the comparative lack of pigment formation in strain ATCC 11078 is a consequence of a greatly reduced rate of biosynthesis of 1.

The removal of oxygen atoms from aromatic and hydroaromatic rings may be of wider importance in biosynthetic processes than commonly assumed. The incorporation of emodinanthrone[10] into islandicin and rubroskyrin has been noted in this sense.[11] Another relevant example is the recently reported precursor relationship of scytalone (3,4-dihydro-3,6,8-trihydroxy-1(2H)naphthalenone) to vermelone (3,4-dihydro-3,8-dihydroxy-1(2H)naphthalenone)[12] and other naphthalene derivatives.[13]

Acknowledgements. We thank C. DuCharme, G. L. Rock, D. Hairsine and H. Schroeder for skilled technical assistance, Dr. Alvin N. Starratt for kindly supplying an authentic sample of zinniol diacetate and the National Science and Engineering Research Council of Canada for financial support.

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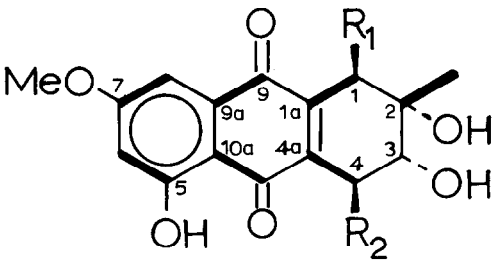
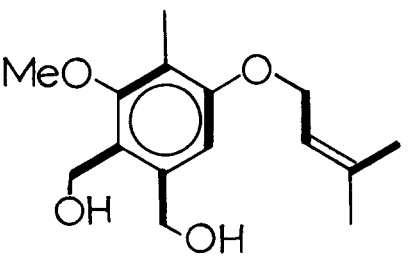


1 $R_1 = R_2 = OH$

2 $R_1 = R_2 = H$

3 $R_1 = OH$ $R_2 = H$



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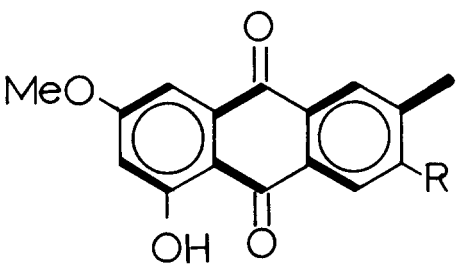

4 R = OH

5 R = H

Table: ^{13}Cmr data[a] for 1, 3 and 4

	1[b]		3[b]		4-OAc$_2$[c]	
	δ_C	J_{CC}	δ_C	J_{CC}	δ_C	J_{CC}
C-1	68.6		69.3		130.1	
		47		47		60
C-1a	142.2		143.0		130.2	
C-2	73.0		72.0		137.0	
		40		41		44
2-Me	22.4		21.9		16.5	
C-3	73.9		66.8		154.3	
C-4	68.7		29.0		120.7	
		44		40		62
C-4a	144.5		144.0		134.0	
C-5	163.3		163.4		152.5	
		69		69		73
C-6	105.9		105.6		115.8	
C-7	165.6		165.6		164.3	
		65		65		67
C-8	106.7		107.0		110.0	
C-9	183.7		182.9		181.7	
		54		53		54
C-9a	133.3		133.5		136.7	
C-10	188.6		188.2		179.6	
		57		57		54
C-10a	109.6		109.1		118.6	
7-OMe	56.3		56.3		56.2	

[a] δ_C in ppm from TMS; J_{CC} values are one-bond ^{13}C-^{13}C couplings in Hz.

[b] In DMSO-d_6.

[c] In $CDCl_3$.

(Received in USA 2 April 1979)