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CONVERSION OF (2*Z*,4*E*)-5-(1′,2′-EPOXY-2′,6′,6′-TRIMETHYLCYCLOHEXYL)-3-METHYL-2,4-PENTADIENOIC ACID TO XANTHOXIN ACID BY *CERCOSPORA CRUENTA*, A FUNGUS PRODUCING (+)-ABSCISIC ACID

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Key Word Index—*Cercospora cruenta*; fungus; metabolism; plant growth inhibitor; xanthoxin acid; abscisic acid.

Abstract—(±)-(2*Z*,4*E*)-5-(1′,2′-epoxy-2′,6′,6′-trimethylcyclohexyl)-3-methyl-2,4-pentadienoic acid was metabolized by *Cercospora cruenta*, which has the ability to produce (+)-abscisic acid (ABA), to give (±)-(2*Z*,4*E*)-xanthoxin acid, (±)-(2*Z*,4*E*)-5′-hydroxy-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetic acid, (±)-1′,2′-epoxy-1′,2′-dihydro-β-ionone and trace amounts of ABA.

INTRODUCTION

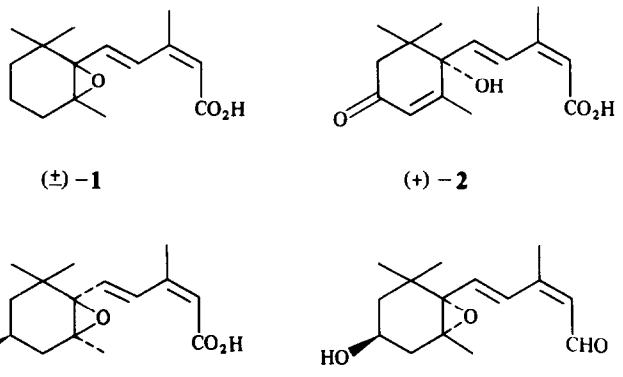
The (+)-(1′*S*,2′*R*)-epoxy acid (**1**) has a similar growth inhibitory activity as ABA (**2**) [1, 2]. Milborrow *et al.* reported that one enantiomer of (±)-(1′*SR*,2′*RS*)-**1** is metabolized to (+)-ABA and the other enantiomer is converted to (−)-(2*Z*,4*E*)-(1′*R*,2′*S*,4′*S*)-1′,2′-epi-xanthoxin acid (**3**) by plants [3]. The latter (**3**) was not converted into ABA. Firn *et al.* reported that (−)-(1′*S*,2′*R*,4′*S*)-xanthoxin (**4**), identified in plant extracts, is converted to (+)-(*S*)-ABA in plant tissue [4]. The fungus *Cercospora cruenta* IFO 6164, which can produce relatively high levels of (+)-ABA in culture broth [5], was used to examine the metabolism of (±)-**1**. In this paper we report the identification of the metabolities formed from (±)-**1** by *C. cruenta*.

RESULTS AND DISCUSSION

(±)-Epoxy acid (**1**) was metabolized by the suspension of mycelia of *C. cruenta*. The separated acidic metabolites were analysed by TLC (silica gel, solvent system A; benzene–ethyl acetate–acetic acid, 40:10:1) to show five components (R_f: 0.13 for **7a**, 0.18 for **8a**, 0.32 for **5a**, 0.36 for **9a** and 0.46 for **1**) [5]. The separation of the metabolites by preparative TLC gave one crystalline acid (**7a**), mp 202–203° (R_f 0.13). (±)-1′,2′-Epoxy-1′,2′-dihydro-β-ionone (**6**) [1, 2] was separated from the neutral metabolites. The acidic metabolites were methylated with diazomethane and then separated by TLC into individual components (solvent system B; benzene–ethyl acetate, 4:1, R_f: 0.15 for **7b**, 0.24 for **8b**, 0.46 for **5b** and 0.85 for **1** methyl ester).

Identification of the metabolites

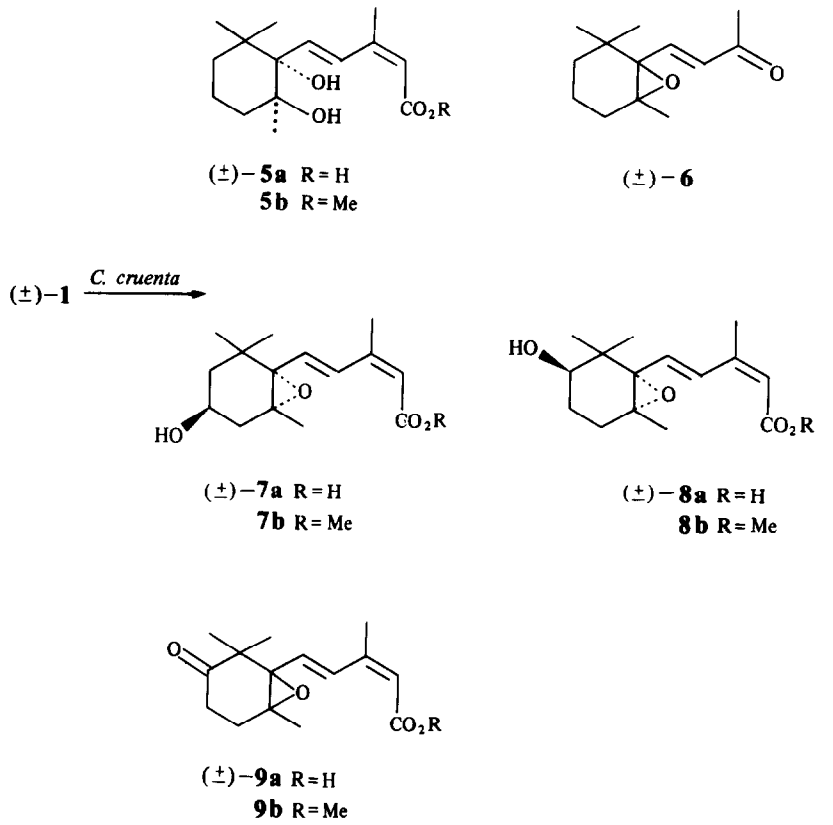
The ^{1}H NMR spectrum of **5b**, mp 149°, $[\alpha]_D$ 0° (EtOH), was identical with that of (±)-(2*Z*,4*E*)-methyl *trans*-1′,2′-dihydroxy-1′,2′-dihydro-β-ionylideneacetate, which was prepared by the treatment of (±)-**1** methyl ester with methanolic sulphuric acid [1, 2]. The MS of **7b**,



(±)-**1** (+)-**2**

3 **4**

Scheme 1.



(±)-**5a** R=H
5b R=Me

(±)-**6**

(±)-**7a** R=H
7b R=Me

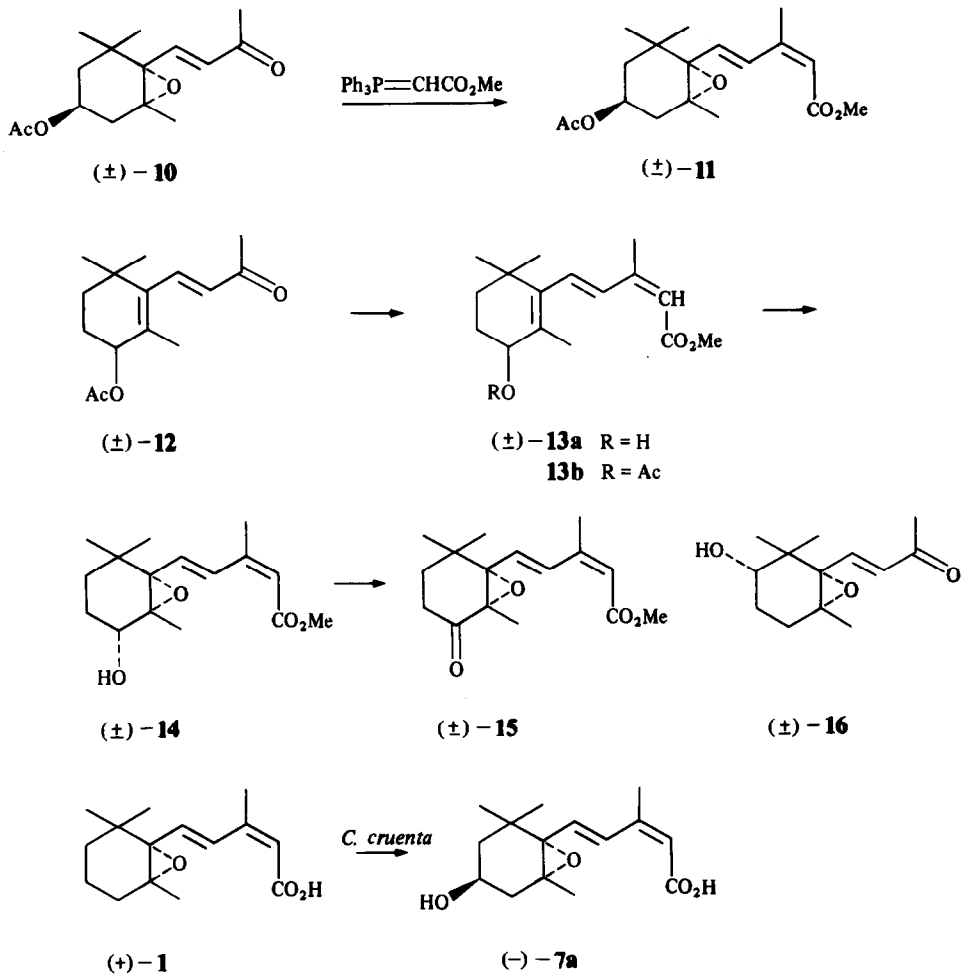
(±)-**8a** R=H
8b R=Me

(±)-**9a** R=H
9b R=Me

Scheme 2.

mp 106–107°, $[\alpha]_D$0° ($CHCl_3$), exhibited peaks at *m/z*: 280 $[M]^+$, 265, 248, 222, 207, 180, 147, 125, 123, 119, 106. The presence of methyl 3-methyl-2,4-pentadienoate moiety in **7b** was suggested from a peak at *m/z* 125 and the UV absorption at 265 nm (λ_{max}). An M_r of 280 ($C_{16}H_{24}O_4$) suggested that **7b** contains one more oxygen, as a hydroxyl group (ν_{max}: 3440 cm^{-1}), than **1** methyl ester. The NMR spectrum of **7b** showed the presence of three methyl groups on the cyclohexane ring at δ1.01 (3H), 1.15 (3H), 1.23 (3H) like those of **1** methyl ester (δ0.97 (3H), 1.10 (3H), 1.18 (3H) [1]) and methyl 3-methyl-(2*Z*,4*E*)-pentadienoate moiety at δ5.70 (1H), 6.28 (1H, *d*, *J* = 16 Hz), 7.61 (1H, *d*, *J* = 16 Hz), 2.01 (3H), 3.70 (3H). The ^{1}H NMR spectrum of the monoacetate, obtained by the treatment of **7b** with acetic anhydride–pyridine, showed the presence of an AcO–C–H group at δ2.04 (3H) and 4.94 (1H). Oxidation of **7b** with chromium trioxide in pyridine gave (±)-ABA methyl ester, $[\alpha]_D$0° (EtOH), the NMR spectrum of which was identical with that of an authentic specimen. This result means that the hydroxyl group is present at the 4′-position on the cyclohexane ring of **7b**. The MS of **8b**, $[\alpha]_D$0° (EtOH), showed peaks at *m/z*: 280 $[M]^+$, 265, 262, 221, 205, 154, 125, 121, 105. An M_r of 280 ($C_{16}H_{24}O_4$) suggested that **8b** contains one more oxygen, as a hydroxyl group (ν_{max}: 3500 cm^{-1}), than **1** methyl ester. The ^{1}H NMR spectrum of **8b** exhibited the presence of a methyl 3-methyl-(2*Z*,4*E*)-pentadienoate moiety at δ5.70 (1H), 6.24 (1H, *d*, *J* = 16 Hz), 7.58 (1H, *d*, *J* = 16 Hz), 2.02 (3H) and 3.70 (3H), and three methyl groups at δ1.06 (3H), 1.07 (3H) and 1.18 (3H) on the cyclohexane ring. The UV spectrum of **8b** showed an absorption at 266 nm (λ_{max}) caused by the 2,4-pentadienoate system. The ^{1}H NMR spectrum of **8b** monoacetate showed the presence of AcO–C–H at δ2.05 (3H) and 4.78 (1H, *br q*, *J* = 4 Hz, $W_{1/2}$ = 16 Hz, *axial*). Oxidation of **8b** with chromium trioxide in pyridine gave the keto-ester (**9b**), which was identical with the compound corresponding to R_f 0.46 by co-chromatography on TLC (yellow colour by heating after spraying with 5% sulphuric acid). Therefore, the above data suggested that the hydroxyl group in **8b** is attached to the C-3′ or C-5′ of the cyclohexane ring.

In order to elucidate the stereochemistry of the hydroxyl groups in **7b** and **8b**, the compounds with 3′-hydroxyl and 4′-acetoxy groups on the cyclohexane ring of (±)-**1** were synthesized. The Wittig reaction of (±)-*trans*-4′-acetoxy-epoxide (**10**) [6] with methoxycarbonylmethylenetriphenylphosphorane gave a stereoisomeric mixture of (±)-(2*Z*,4*E*) and (2*E*,4*E*)-4′-*O*-acetylxanthoxin acid methyl esters, which was separated by preparative TLC to give the (2*Z*,4*E*)-ester (**11**), mp 80.5° and the (2*E*,4*E*)-isomer, mp 92.5°. The ^{1}H NMR spectrum of **11** was identical with that of the acetate of **7b**. Also, the reaction of (±)-3′-acetoxy-β-ionone (**12**) [1] with the phosphorane gave a stereoisomeric mixture of (±)-(2*Z*,4*E*) and (2*E*,4*E*)-methyl 3′-acetoxy-β-ionylideneacetates (**13b**), which were deacetylated and then epoxidized with *m*-chloroperbenzoic acid to give a stereoisomeric mixture of (±)-(2*Z*,4*E*)- and (2*E*,4*E*)-methyl 3′-hydroxy-1′,2′-dihydro-β-ionylideneacetates, which were separated by



Scheme 3.

preparative TLC to give (±)-(2*Z*,4*E*)-hydroxy ester (**14**). Oxidation of **14** with chromium trioxide in pyridine gave (±)-(2*Z*,4*E*)-methyl 3′-oxo-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetate (**15**). The ^{1}H NMR spectrum of **15** was different from that of **9b**. This means that the hydroxyl group in **8b** is located at the 5′-position of the cyclohexane ring. Ohta *et al.* reported the synthesis of (1′*R**,2′*S**,5′*R**)-(3*Z*)-4-(5′-hydroxy-2′,6′,6′-trimethyl-1′,2′-epoxy-1′-cyclohexyl)-3-buten-2-one (**16**) and its ^{1}H NMR data [7]. As the signal pattern of 2′,6′,6′-trimethyl protons (δ1.06, 1.07 and 1.18) and the 5′-methyne proton (δ3.68) on the cyclohexane ring of **8b** are different from those of the corresponding protons (δ1.02, 1.12, 1.14 and 3.13) in **16** on their ^{1}H NMR spectra, it is suggested that the 5′-hydroxyl group of **8b** is *trans* to the epoxy ring.

Further, it was confirmed that the above acidic metabolites, **5a**, **7a**, **8a** and **9a**, were formed from the precursor (±)-**1** by *C. cruenta*. (±)-[2-^{14}C]-Epoxy acid (**1**) was metabolized by *C. cruenta* to acidic metabolites, which were methylated with diazomethane and then separated by preparative TLC into each component. The incorporation of the radioactivity into the acidic metabolites are shown in Table 1. The separated ABA methyl ester were counted as the corresponding *cis* and *trans*-1′,4′-diol methyl esters obtained by sodium borohydride reduction according to Milborrow's method [3]. The incorporation of (±)-[2-^{14}C]-**1** into ABA (0.6%) was low and the detection of ABA in the cold acidic metabolites was difficult [5]. The amount of ABA contained in the broth of *C. cruenta* cultured by the addition of (±)-**1** (1–5 mg/100 ml) was decreased to 14–15% compared with

Table 1. Incorporation of (±)-2-^{14}C-epoxy acid (**1**) (30 mg, 405.0 × 10^3 dpm) into acidic metabolites by *Cercospora cruenta* (1 l.)

Metabolites	Radioactivity (10^{-3} × dpm)	Incorporation ratio (%)
Recovered (±)-**1**	92.9	
Dihydroxy acid Me ester (**5b**)	68.0	21.8
Xanthoxin acid Me ester (**7b**)	95.8	30.7
5′-Hydroxy-epoxy acid Me ester (**8b**)	88.0	28.2
5′-Oxo-epoxy acid Me ester (**9b**)	11.7	3.8
ABA Me ester	1.8	0.6

that of the control by GC analysis. Therefore, it appears from these that the biosynthesis of ABA in *C. cruenta* is inhibited by (±)-**1**. As the treatment of (±)-**1** with the boiled culture broth did not give the dihydroxy-acid (**5a**), **5a** is probably formed by the enzymic hydrolysis of (±)-**1** with *C. cruenta*. Similarly, the microbial oxidation of (+)-(1′*S*,2′*R*)-**1** and (−)-(1′*R*,2′*S*)-**1** by *C. cruenta*, followed by the treatment with diazomethane, gave (−)-(1′*S*,2′*R*,4′*S*)-(2*Z*,4*E*)-xanthoxin acid methyl ester (**7b**), mp 134–135°, and (+)-(1′*R*,2′*S*,4′*R*)-enantiomer, mp 135–136° [8], respectively.

From the above result, it is obvious that (±)-epoxy acid (**1**) is non-enantioselectively oxidized by *C. cruenta* to give (±)-xanthoxin acid (**7a**), in contrast to the enantioselective metabolism of the (±)-**1** in plants [3].

EXPERIMENTAL

Metabolism of (±)*-epoxy acid* (**1**) *by* Cercospora cruenta *IFO* 6164. *Cercospora cruenta* was subcultured in 1 l. of the liquid medium, prepared from a 500 ml of potato medium and 500 ml Miller medium, under shaking and lighting (1500 lux) at 28° for 6 days. The mycelium was collected by centrifugation at 11 000 *g*, washed with an M/30 phosphate buffer (pH 7.4) and was then suspended in 1 l. of Czapek-Dox medium without glucose. To the suspension was added 50 mg of (±)-**1** (as an aqueous sodium salt). After incubation for 3 days, the culture broth was filtered, and the filtrate was extracted with EtOAc at pH 2.5. Extraction of the organic layer with 5% aq. $NaHCO_3$ followed by acidification and re-extraction gave an acidic fraction, which was methylated with ethereal CH_2N_2 to give the methyl ester fraction (46.3 mg). The majority of the latter fraction consisted of five compounds as determined by TLC (silica gel, Merck H, 0.25 mm thick, solvent system B; C_6H_6–EtOAc, 4:1, R_f: 0.15 for **7b**, 0.24 for **8b**, 0.46 for **5b**, 0.56 for **9b** and 0.85 for 1 methyl ester). The methyl ester fraction was separated by prep. TLC (silica gel, Merck PF_{254}, solvent system B) twice to give each component, 7.2 mg of **7b**, 7.0 mg of **8b**, 2.9 mg of **5b**, 1.5 mg of **9b** and 10.4 mg of the recovered (±)-**1** methyl ester. Similarly, the acidic fraction (2.5 mg) was separated from the suspension (1 l.) of mycelia of *C. cruenta* without (±)-**1** incubated for 3 days and then treated with CH_2N_2 to give the control methyl ester fraction, which did not contain the above methyl esters, **5b**, **7b**, **8b** and **9b** by TLC analysis. Separation of the neutral fraction by prep. TLC gave 1.1 mg of (±)-1′,2′-epoxy-1′,2′-dihydro-β-ionone (**6**), $[\alpha]_D^{20}$ 0° ($CHCl_3$), which was not detected in the control neutral fraction by TLC analysis.

(±)*-Xanthoxin acid methyl ester* (**7b**). The crystalline ester (**7b**) separated from the esterified metabolites of (±)-**1** showed mp 106–107°C (from hexane–C_6H_6). UV λ_{max}^{EtOH} 265 nm; IR ν_{max} cm^{-1}: 3440, 1720, 1630, 1600, 1230, 1160, 1045, 990. EIMS *m/z* (rel. int.): 280 $[M]^+$ (8), 265 (8), 248 (14), 222 (13), 207 (8), 180 (18), 147 (23), 125 (16), 123 (41), 119 (33), 106 (20), 43 (100). ^1H NMR (100 MHz, $CDCl_3$): δ1.01 (3H, *s*), 1.15 (3H, *s*), 1.23 (3H, *s*), 1.57 (1H, *s*, OH), 2.01 (3H, *d*, *J* = 1 Hz), 3.70 (3H, *s*), 3.93 (1H, *m*), 5.70 (1H, *s*), 6.28 (1H, *d*, *J* = 16 Hz), 7.61 (1H, *d*, *J* = 16 Hz). GC analysis with a 2 m × 3 mm stainless steel column packed with 5% SE-30 (FID), column temp. 200°, N_2 flow rate 18 ml/min, retention time (R_t): 10.6 min (one peak). Oxidation of **7b** with CrO_3 in pyridine overnight at room temp. gave (±)-ABA methyl ester, $[\alpha]_D$ 0° (EtOH). Acetylation of **7b** with Ac_2O–pyridine overnight at room temp. gave the monoacetate of **7b**. ^1H NMR ($CDCl_3$): δ1.04 (3H, *s*), 1.18 (3H, *s*), 1.24 (3H, *s*), 2.04 (6H), 3.80 (3H, *s*), 4.94 (1H, *m*, $W_{1/2}$ = 8 Hz), 5.68 (1H, *m*), 6.17 (1H, *d*, *J* = 16 Hz), 7.60 (1H, *d*, *J* = 16 Hz).

Synthesis of (±)-(2Z,4E)-4′-O-*acetyl-xanthoxin acid methyl ester* (**11**). The reaction of (±)-*trans*-4′-acetoxy-epoxide (**10**) (1.0 mM) with methoxycarbonylmethylenetriphenylphosphorane (1.5 mM) in dry xylene (7 ml) under reflux for 28 hr gave a stereoisomeric mixture of (±)-(2Z,4E) and (2*E*,4*E*)-esters (85% yield), which was separated by TLC to give (±)-(2*Z*,4*E*)-ester (**11**), mp 80.5° and (±)-(2*E*,4*E*)-isomer, mp 92.9°. The ^1H NMR spectrum of **11** was identical with that of the monoacetate of **7b**. ^1H NMR of the (2*E*,4*E*)-isomer ($CDCl_3$): δ1.00 (3H, *s*), 1.19 (3H, *s*), 1.25 (3H, *s*), 2.04 (3H, *s*), 2.32 (3H, *s*), 3.73 (3H, *s*), 4.92 (1H, *m*), 5.80 (1H, *s*), 6.26 (1H, *s*), 7.24 (1H, *s*).

(±)-(2Z,4E)-*Methyl 5′-hydroxy-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetate* (**8b**). The oily ester (**8b**) was separated from the esterified metabolites of (±)-**1**. GC analysis with the same column and column conditions as used for **7b** showed R_t 11.2 min (one peak) for **8b**. EIMS *m/z* (rel. int.): 280 $[M]^+$ (9), 265 (11), 262 (6), 221 (15), 205 (25), 154 (67), 149 (57), 125 (52), 121 (100), 105 (53). UV λ_{max}^{EtOH} 266 nm; IR ν_{max}^{film} cm^{-1}: 3500, 1720, 1635, 1600, 1240, 1170, 1050, 990; ^1H NMR ($CDCl_3$): δ1.06 (3H, *s*), 1.07 (3H, *s*), 1.18 (3H, *s*), 1.94 (1H, *s*, OH), 2.02 (3H, *d*, *J* = 1 Hz), 3.68 (1H, *m*), 3.70 (3H, *s*), 5.70 (1H, *s*), 6.24 (1H, *d*, *J* = 16 Hz), 7.58 (1H, *d*, *J* = 16 Hz). Acetylation of **8b** with Ac_2O–pyridine overnight gave the monoacetate of **8b**. ^1H NMR ($CDCl_3$): δ1.00 (3H, *s*), 1.10 (3H, *s*), 1.20 (3H, *s*), 2.01 (3H, *s*), 2.05 (3H), 3.70 (3H, *s*), 4.78 (1H, *q*, *J* = 4 Hz, $W_{1/2}$ = 16 Hz), 5.72 (1H, *s*), 6.22 (1H, *d*, *J* = 16 Hz), 7.61 (1H, *d*, *J* = 16 Hz). Oxidation of **8b** with CrO_3 in pyridine gave (±)-(2Z,4E)-methyl 5′-oxo-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetate (**9b**), $[\alpha]_D^{20}$ 0° (EtOH). IR ν_{max}^{film} cm^{-1}: 1720, 1640, 1610, 1230, 1165; ^1H NMR ($CDCl_3$): δ1.16 (3H, *s*), 1.25 (3H, *s*), 1.33 (3H, *s*), 2.04 (3H, *s*), 2.20–2.40 (4H, *m*), 3.71 (3H, *s*), 5.76 (1H, *s*), 6.19 (1H, *d*, *J* = 16 Hz), 7.69 (1H, *d*, *J* = 16 Hz), UV λ_{max}^{EtOH} 264 nm. The above keto ester (**9b**) was identical with the compound corresponding to R_f 0.56 in the esterified metabolites of (±)-**1** by co-chromatography on the TLC (the same R_f value and yellow colour by heating after spraying with 5% H_2SO_4).

Synthesis of (±)-(2Z,4E)-*methyl 3′-hydroxy-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetate* (**14**). The reaction of (±)-3′-acetoxy-β-ionone (**11**) (1.0 mM) with methoxycarbonylmethylenetriphenylphosphorane (1.5 mM) in 5 ml of dry xylene under reflux for 2 days, followed by the column chromatography with silica gel, gave a stereoisomeric mixture of (±)-(2*Z*,4*E*)-3′-acetoxy-β-ionylideneacetates (**13b**) (80% yield); IR ν_{max}^{film} cm^{-1}: 1745, 1730, 1605, 1240, 1160, 1015, 970. The acetoxy esters (**13b**) were hydrolysed with 5% ethanolic NaOH and then methylated with ethereal CH_2N_2 to give the hydroxy-esters (**13a**), which were epoxidized with *m*-chloroperbenzoic acid in $CHCl_3$ overnight at 5°. The products were separated by the TLC to give (±)-(2*Z*,4*E*)-methyl 3′-hydroxy-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetate (**14**) as an oil. IR ν_{max}^{film} cm^{-1}: 3480, 1720, 1640, 1605, 1240, 1160, 1050, 990; ^1H NMR ($CDCl_3$): δ1.00 (3H, *s*), 1.09 (3H, *s*), 1.32 (3H, *s*), 2.02 (3H, *d*, *J* = 1 Hz), 2.50 (1H, *s*, OH), 5.73 (1H, *s*), 6.23 (1H, *d*, *J* = 16 Hz), 7.64 (1H, *d*, *J* = 16 Hz); ^1H NMR ($CDCl_3$) of the (2*E*,4*E*)-isomer: δ1.03 (3H, *s*), 1.17 (3H, *s*), 1.37 (3H, *s*), 2.35 (3H), 3.75 (3H, *s*), 5.83 (1H, *s*), 6.27 (2H, *s*). Oxidation of **14** with CrO_3 in pyridine gave (±)-(2*Z*,4*E*)-methyl 3′-oxo-1′,2′-epoxy-1′,2′-dihydro-β-ionylideneacetate (**15**) as an oil. IR ν_{max}^{film} cm^{-1}: 1720, 1640, 1610, 1240, 1165, 990; ^1H NMR ($CDCl_3$): δ1.07 (3H, *s*), 1.14 (3H, *s*), 1.30 (3H, *s*), 2.04 (3H, *d*, *J* = 1 Hz), 2.30–2.50 (2H, *m*), 3.71 (3H, *s*), 5.76 (1H, *s*), 6.21 (1H, *d*, *J* = 16 Hz), 7.70 (1H, *d*, *J* = 16 Hz).

(±)-(2Z,4E)-*Methyl 1′,2′-dihydroxy-1′,2′-dihydro-β-ionylideneacetate* (**5b**). The dihydroxy ester (**5b**) was separated from the esterified metabolites of (±)-**1**, mp 148–150°C; ^1H NMR ($CDCl_3$): δ0.85 (3H, *s*), 1.14 (3H, *s*), 1.18 (3H, *s*), 1.68 and 1.74 (2H, 2-OH), 2.06 (1H, *s*, *J* = 1 Hz), 3.70 (3H, *s*), 5.70 (1H, *s*), 6.60 (1H, *dd*, *J* = 1 and 16 Hz).

Metabolism of (+) and (−)-epoxy acids (**1**) *by* C. cruenta. (+)-Epoxy acid (**1**) (50 mg, as an aqueous sodium salt) was added to 1 l. of the suspension of mycelia of *C. cruenta*. After incubation for 2 days, the acidic metabolites were separated and then methylated with ethereal CH_2N_2. The esterified metabolites were separated by the TLC to give 7.0 mg of (−)-(2*Z*,4*E*)-xanthoxin acid methyl ester (**7b**), mp 134–135°; CD $[\theta]_{300}$ −1950, $[\theta]_{265}$ +4670, $[\theta]_{205}$ −31 150 (*c* 0.07%; EtOH) and 3.6 mg of the dihydroxy-ester (**5b**), mp 147–149°. Similarly, the treatment of (−)-epoxy acid (**1**), which was also prepared by us [1], with mycelia of *C. cruenta* gave (+)-(2*Z*,4*E*)-xanthoxin acid methyl ester (**7b**), mp 135–136°, $[\alpha]_D^{20}$ +43° (*c* 1%; $CHCl_3$).

Incorporation of (±)-(2Z,4E)-[2-^{14}C]-*epoxy acid* (**1**) *into acidic metabolites by* C. cruenta. (±)-[2-^{14}C]-Epoxy acid (**1**) (30 mg, total 405.0×10^3 dpm, as an aqueous sodium salt), which was prepared by the reaction of (±)-**6** with [2-^{14}C]-methoxycarbonylmethylenetriphenylphosphorane [1, 2], was added to 1 l. of the suspension of mycelia of *C. cruenta*. After incubation for 3 days, the acidic fraction was separated from the culture broth by the ordinary manner. The recovery of the radioactivity was 90.7% (367.3×10^3 dpm) for the acidic fraction, which was diluted with 1 mg of cold (+)-ABA and then methylated with ethereal CH_2N_2. The methyl ester fraction was separated by prep. TLC to each component, **5b**, **7b**, **8b**, **9b** and ABA methyl ester. The incorporation of radioactivity into these compounds is listed in Table 1. The reduction of the ABA methyl ester with $NaBH_4$ in 70% EtOH, followed by separation by prep. TLC, gave the corresponding *cis*- and *trans*-1′,4′-diol esters [3]. The combined radioactivity of the diol esters was 1.8×10^3 dpm (0.6% incorporation to ABA).

Inhibition of the biosynthesis of ABA in C. cruenta *by* (±)-*epoxy acid* (**1**). *Cercospora cruenta* was subcultured in three portions of the liquid medium (100 ml) for 5 days at 28° (1500 lux). To each of the culture broth were added 2 ml of water (control), 1 mg and 5 mg of (±)-epoxy acid (**1**) as 2 ml of an aq. soln of sodium salt respectively. After incubation for 5 days, acidic metabolites were separated from each of culture broth and then methylated with ethereal CH_2N_2. Each of the methyl ester fractions was analysed by GC with the same column and column conditions as used for **7b**, R_t: 5.8 min for **1** methyl ester, 10.6 min for **5b** and **7b**, 13.3 min for ABA methyl ester. The amounts of ABA methyl ester were 1000 μg (100%) for control [5], 140 μg (14%) for the addition of 1 mg of (±)-**1** and 150 μg (15%) for 5 mg of (±)-**1**.

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REFERENCES

1. Oritani, T. and Yamashita, K. (1983) *Phytochemistry* **22**, 1909.
2. Tamura, S. and Nagao, M. (1969) *Agric. Biol. Chem.* **33**, 296.
3. Milborrow, B. V. and Garmston, M. (1973) *Phytochemistry* **12**, 1597.
4. Firn, R. D., Burden, R. S. and Taylor, H. F. (1972) *Planta* **102**, 115.
5. Oritani, T., Ichimura, M. and Yamashita, K. (1982) *Agric. Biol. Chem.* **46**, 1959.
6. Mori, K. (1973) *Tetrahedron Letters* 2635.
7. Ohta, S., Frei, B. and Jeger, O. (1982) *Helv. Chim. Acta* **65**, 2363.
8. Kienzle, F., Meyer, H., Minder, R. E. and Thommen, H. (1978) *Helv. Chim. Acta* **61**, 2616.