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Structure, Synthesis, and Stereochemistry of (+)-Orthosporin, a Phytotoxic Metabolite of Rhynchosporium Orthosporum

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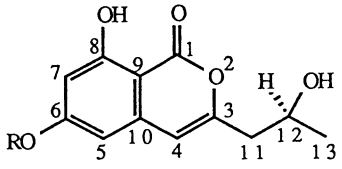
Structure of (+)-orthosporin, a phytotoxic metabolite isolated from Rhynchosporium orthosporum has been elucidated from spectroscopic data and chemical reaction, and the stereochemistry of (+)-orthosporin and (+)-diaporthin has been confirmed by the synthesis.

(+)-Orthosporin (1) is a phytotoxic metabolite isolated from the culture filtrate of Rhynchosporium orthosporum Caldwell, which induces leaf scald on orchard grass. (+)-Orthosporin inhibited the root growth of lettuce (great lake 366) 63.2% at 250 ppm and of the host plant (okamidori) 50.3% at 25 ppm. In present communication, we would like to describe the isolation, structure determination and stereochemical study by the synthesis of this phytotoxin.



1 R=H
2 R=CH_3

The fungus was grown by surface culture on potato-sucrose medium at 25 °C for 30 days in the dark. The culture filtrate (7.5 l) was concentrated in vacuo and the residue was extracted with ethyl acetate. The extracts were purified through silica gel column chromatography, checking with the bioassay of germination of lettuce seeds to give a phytotoxic compound, (+)-orthosporin (1, 11.0 mg).

(+)-Orthosporin (1), needles, mp 183-184 °C, $[\alpha]_D^{22}$+61.8 (c 1.0, CH_3OH), has a molecular formula $C_{12}H_{12}O_5$ from the high resolution MS m/z 236.0700 (M^+, calcd, 236.0685). The UV spectrum showed absorptions at λ nm (ξ) 246 (50455), 279 (7955), 327 (7576). The IR spectrum exhibited absorption bands at ν cm^{-1} 3400 (OH), 1690 (C=O). These spectra revealed the presence of typical isocoumarin moiety in comparison with known isoconmarins.[1] The ^{13}C NMR spectrum showed the presence of one methyl, one methylene, four methines and six quaternary carbons.[2] The ^1H NMR spectrum (Table 1) exhibited signals at δ1.22 (d J=6.1 Hz), 2.56 (dd J=14.3, 7.6 Hz), 2.61 (dd J=14.3, 5.8 Hz) and 4.17 (m) ascribable to 2-hydroxypropyl moiety.

The tetra-substituted aromatic moiety was deduced by the fact that the signals at δ 6.36 (J=2.1 Hz) and 6.40 (J=2.1 Hz) due to two aromatic protons (5-ArH, 7-ArH) shows a meta coupling.

Treatment of 1 with diazomethane gave quantitatively a methylated compound 2, mp 78-81 °C, [α] +55 (c 0.9, $CHCl_3$), $C_{13}H_{14}O_5$, from the high resolution MS m/z 250.0869 (M^+, calcd, 250.0842), whose spectral data[3] are in accord with those of

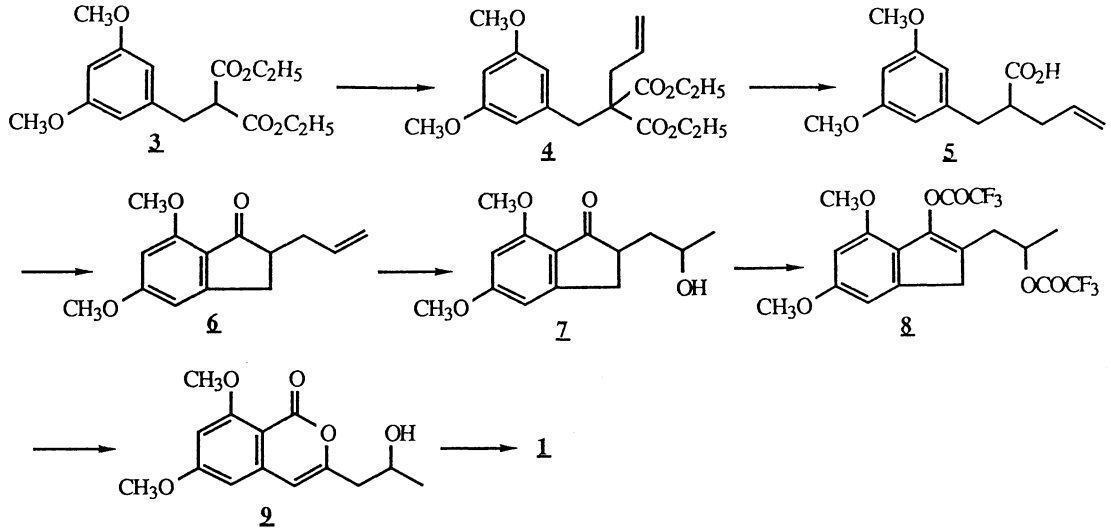
Table 1. ^1H NMR (400 MHz, acetone-d_6) spectrum of (+)-orthosporin (1)

Chemical shift (δ)	Number of proton	Multiplicity (Hz)	Assignment
1.22	3H	d,J=6.1	13-CH_3
2.56	1H	dd,J=14.3, 7.6	11-CH_A
2.61	1H	dd,J=14.3, 5.8	11-CH_B
3.86	1H	S	6-ArOH
3.97	1H	S	12-OH
4.17	1H	m	12-CH
6.36	1H	d,J=2.1	5-ArH
6.40	1H	d,J=2.1	7-ArH
6.41	1H	S	4-CH

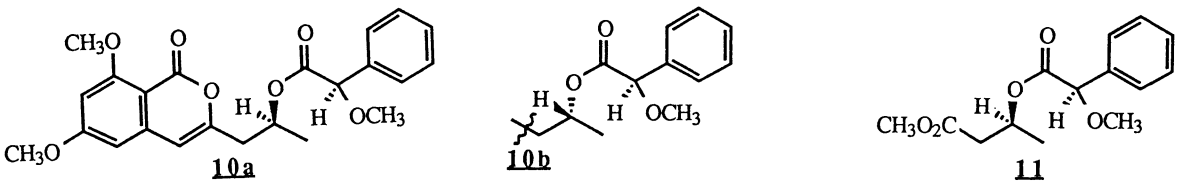
(+)-diaporthin (2), mp 83-85 °C, $[\alpha]_D$ +54 (c 0.87, $CHCl_3$), a phytotoxin isolated from the culture filtrate of Endothia parasitica, a parasite of chestnut.[4] From the results described above, the planar structure of (+)-orthosporin (1) has been confirmed to be 3-(2-hydroxypropyl)-6,8-dihydroxyisocoumarin.

Absolute configuration of (+)-orthosporin (1) and (+)-diaporthin (2) has been determined by the synthesis, since natural sample (1) available is limited. The diester 3[5] (21.3 mmol) was alkylated with allyl bromide (26.3 mmol) in the presence of sodium ethoxide by refluxing in ethanol to give an alkylated product 4[6] (80.6%), mp 63.5-64.0 °C, which was hydrolysed to a carboxylic acid 5[7] (97.6%) with potassium hydroxide in water-dioxane containing 18-crown-6. The acid 5 was converted to an indanone 6[8] (89.1%), mp 52.5—53 °C, with trifluoroacetic anhydride under ice-cooling. Oxymercuration of the indanone (0.47 mmol) with mercuric acetate (0.47 mmol) in water-THF at room temperature, and subsequent demercuration with sodium borohydride (0.19 m mol) yielded an alcohol 7[9] (58.7%). The alcohol 7 was treated with trifluoroacetic anhydride to give a trifluoroacetate 8,[10] which was subjected to ozonolysis in ethyl acetate at -70 °C, and then decomposition of the ozonide with dimethyl sulfide to yield an isocoumarin 9[11] (83.5% from 7). Removal of protecting group of 9 (0.064 mmol) with aluminum chloride (0.52 mmol) in ethanethiol afforded (±)-orthosporin (1), mp 182-183 °C, whose spectral data were identical with those of natural sample.

In order to determine the absolute configuration, optical resolutions were attempted with (±)-1 and the racemic intermediates. Though direct resolution of (±)-9, (±)-2 and (±)-1 using chiral colunm CHIRALCEL OC gave no clear separation, (±)-9 was separated partially to two peaks using CHIRALCEL OD. However, being difficulty obtaining adequate amount of optical isomers, optical resolution with chiral column was abandoned. Successful results were obtained by the resolution with a diastereomeric mixture, 10a and 10b, which was prepared by esterification of (±)-9 (0.21 mmol) with (R)-2-methoxy-2-phenylacetyl chloride (0.53 mmol). Thus, by PTLC (kieselgel 60 F_{254}) eluting with benzene-ethyl acetate (1:1 v/v), the mixture was partially separated to 10a (Rf 0.47) and 10b (Rf 0.42),[12] which



were converted to (+)-9, $[\alpha]_D$ +22.4, (c 2.7 $CHCl_3$) and (-)-9, $[\alpha]_D$ -39.7 (c 0.14, $CHCl_3$) through hydrolysis. Removal of methyl group in each of these enantiomers with aluminum chloride in ethanethiol gave (+)-1, $[\alpha]_D$ +17.5 (c 1.3, CH_3OH) and (-)-1, $[\alpha]_D$ -22.4 (c 0.22, CH_3OH) respectively. Since natural orthosporin (1) shows optical rotation $[\alpha]_D$ +61.8, the enatiomers 10a, (+)-9 and (+)-1 have the same absolute configuration with that of (+)-orthosporin (1). On the other hand, ozonolysis of 10a in methanol at -70 °C and subsequent decomposition of ozonide with dimethyl sulfide yielded a yellow oil which was methylated with diazomethane to give an ester 11. Comparison of the ^1H NMR spectrum of the ester 11 with those of (R)-11 and (RS)-11 which were prepared from (R)- and (RS)-methyl 3-hydroxybutyrates through acylation with (R)-2-methoxy-2-phenylacetyl chloride, revealed that the signals of 11 are in fair agreement with (S)-11 (Table 2).[13]



Therefore, absolute configuration of natural (+)-orthosporin should be (S)-configuration as shown in 1. Very recently, the same structure with 1 has been suggested for a phytotoxin, de-O-methyldiaporthin, from Drechslera siccans, though no stereochemical evidence has been revealed.[14]

References

1) M.A.W.Eaton and D.W.Hutchinson, Tetrahedron Lett., 1971,1337.

2) 1, ^{13}C NMR (25.1 MHz) δ 23.6 (13-CH_3), 43.9 (11-CH_2), 65.5 (12-CH), 99.7 (9-C), 102.2 (7-CH), 103.2 (5-CH), 106.3 (4-CH), 140.8 (10-C), 156.3 (3-C), 164.5 (8-C), 166.2 (6-C), 166.9 (1-C).

3) Synthetic 2: IR ν cm^{-1} 3450, 1690, 1630, ^1H NMR (100 MHz); δ1.31 (3H, d, J=6.3 Hz, 13-CH_3), 2.61 (2H, d, J=4.3 Hz, 11-CH_2), 3.87 (3H, S, OCH_3), 3.96 (1H, S, 12-OH), 4.28 (1H, m, 12-CH), 6.28 (1H, s, 4-CH), 6.33 (1H, d, J=2.2 Hz 5-CH), 6.47 (1H, d, J=2.2 Hz, 7-CH), 11.04 (1H, s, 8-OH).

4) A. Boller, E. Gauman, E. Hardegger, F. Kugler, St. Naef-Roth, and M. Rosner, Helv. Chim. Acta, 40, 875 (1957); E. Hardegger, W. Rieder, A. Walser, and F. Kugler, ibid., 49, 1283 (1966).

5) R.H.Carter, R.M.Colyer, R.A.Hill, and J. Staunton, J. Chem. Soc., Perkin Trans.1, 1976, 1438.

6) 4, IR ν cm^{-1} 1730; ^1H NMR (90 MHz), 2.51 (2H, d, J=7 Hz, =CH-CH_2), 5.05 (2H, m, =CH_2), 5.57 (1H, ddt, J=16, 9, 7 Hz, =CH).

7) 5, IR ν cm^{-1} 3500-2500, 1700, ^1H NMR (90 MHz), 9.33 (1H, s, COOH).

8) 6, IR ν cm^{-1} 1700; ^1H NMR (90 MHz) δ 1.9-2.3 (1H, m, COCH).

9) 7, IR ν cm^{-1} 3400, 1680; ^1H NMR (90 MHz) δ1.18 (3H, d, J=7 Hz, CH_3), 3.00 (1H, br.s, OH), 3.00-3.40 (1H, m, CHO-).

10) 8, IR ν cm^{-1} 1800, 1780; ^1H NMR (90 MHz) δ 1.32 (3H, d, J=8 Hz, CH_3), 5.15 (1H, tq, J=8.8 Hz, CH).

11) 9, IR ν cm^{-1} 3400, 1690; ^1H NMR (90 MHz), (1H, s, OH), 4.0-4.4 (1H, m, CHO-), 6.13 (1H, s, =CH).

12) 10a, $C_{23}H_{24}O_7$ from HR-MS m/z 412.1522 (calcd, 412.1522); IR ν cm^{-1} 1720; ^{1} NMR (100 MHz) δ 1.23 (3H, d, J=6.1 Hz, CH_3), 4.73 (1H, s, CHO-), 6.02 (1H, s, =CH), 10b, $C_{23}H_{24}O_7$ from HR-MS m/z 412.1550 (calcd, 412.1522); IR $\nu$$cm^{-1}$ 1720; ^1H NMR (100 MHz) δ1.36 (3H, d, J=6.4 Hz, CH_3), 4.70 (1H, s, CHO-), 5.69 (1H, s, =CH).

13) Table 2. Comparison of ^1H NMR spectra (100 MHz, $CDCl_3$) of the ester 11 and its diastereomers, (RS)-11 and (R)-11

11	(RS)-11	(R)-11
1.19 (3H, d, 6.4 Hz)	1.19 (1.5 H, d, 6.3 Hz)	
	1.32 (1.5 H, d, 6.4 Hz)	1.32 (3H, d, 6.1 Hz)
2.56 (1H, d, 5.9 Hz)	2.4-2.6 (2H, m)	2.46 (1H, d, 6.1 Hz)
2.61 (1H, d, 7.3 Hz)		2.50 (1H, d, 7.8 Hz)
3.41 (3H, s)	3.40 (4.5 Hz, s)	3.40 (6H, s)
3.62 (3H, s)	3.61 (1.5 H, s)	
	4.72 (0.5 H, s)	4.71 (1H, s)
4.73 (1H, s)	4.73 (0.5 H, s)	
5.1-5.5 (1H, m)	5.1-5.5 (1H, m)	5.1-5.5 (1H, m)
7.2- 7.5 (1H, m)	7.2-7.5 (1H, m)	7.2-7.5 (1H, m)

14) Y. F. Hallock, J. Clardy, D. S. Kenfield, and G. Strobel, Phytochemistry, 27, 3123 (1988). De-O-methyldiaporthin, white solid, showed $[\alpha]_D$+22 (MeOH, c 0.09).

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