Stereochemistry and Biological Activity of Phytoalexin “Safynol” from Safflower

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The absolute configuration of a phytoalexin “Safynol” (3E,11E)-3,11-tridecadiene-5,7,9-triyne-1,2-diol (I), separated from Phytophthora drechsleri-infected safflower (Carthamus tinctorius L.) was confirmed. (R) and (S)-safynol were synthesized from 2,3-O-isopropylidene-(S)- and (R)-glyceraldehyde respectively, and the (R) configuration was assigned to the natural product. The inhibitory effects of (R)- and (S)-safynol on mycelial growth of five fungi were almost the same and their ED₅₀ values ranged from 6 to 70 ppm.

A wide variety of phytoalexins have been isolated from various plants, however, few reports have concerned the phytoalexin originated from Compositae family. In the study of naturally occurring acetylenes from this family, Bohlmann et al. separated a compound from safflower (Carthamus tinctorius L.)¹) and Centaurea ruthenica L.,²) and established their structure as (3E,11E)-3,11-tridecadiene-5,7,9-triyne-1,2-diol (I), of which the racemic form, was also synthesized by the same authors.³) Later, Allen et al. reported⁴⁻¹⁰) that Phytophthora drechsleri infected-safflower accumulates a large amount of I and their dehydroderivative II. They also observed that I and II show strong growth inhibitory effect on the pathogenic fungi and claimed that I and II are phytoalexins, designating safynol (I) and dehydrosafynol (II) respectively. Bohlmann had reported a levorotation of I but no description appeared on the absolute configuration of safynol and dehydrosafynol. Therefore, we attempted to synthesize optically active I from the starting materials of the known configuration and to establish the absolute configuration of safynol. Furthermore the antifungal activity of the enantiomeric products were compared in the hope to reveal the correlation of the biological activity and the absolute configuration of safynol.

The synthetic process is shown in Fig. 1. For the synthesis of (S) and (R)-safynol, C₃ (IIIa,b), C₄ (IV) and C₇ (X) synthons can be conceived as the disconnection products, and (R) and (S)-2,3-O-isopropylidene-glyceraldehyde (IIIa) and (IIIb) were chosen as the first C₃-synthon to establish the absolute configuration. (R)-2,3-O-Isopropylidene-glyceralde-
aldehyde (IIIa) was prepared by NaIO₄-NaHCO₃ oxidation of 1,2,5,6-di-O-isopropylidene-D-mannitol. To prepare another C₃-synthon, 3-trimethylsilyl-2-propynol was brominated with Ph₃PBr₂ to yield 3-bromo-1-trimethylsilyl-1-propyne (IV). The ylid derived from the triphenylphosphonium salt V was allowed to react with IIIa. The configuration of newly formed double bond was dependent on the reaction temperature. The (Z):(E) ratio of the products analyzed by GLC was 3:1 at -40°C, 4:3 at -20°C and 6:5 at 0°C, but the desirable (E)-isomer could not be obtained in preponderance.

In spite of having the same chiral center in their molecules, the (E) and (Z) dioxolanes, VIa and VIa', showed opposite trend in their ORD curves in the visible spectrum. Cleavage of C-Si bond of VI by silver nitrate resulted in the precipitation of ethynyl silver salt, which was dissolved in dichloromethane without any purification. By the addition of iodine to this solution silver iodide was precipitated and from the organic layer (S)-(E and Z)-2,2-dimethyl-4-(4-iodo-1-buten-3-ynyl)-1,3-dioxolane (VII) was obtained in good yield.

The third synthon, C₇-ethynyl compound X, was prepared following the Bohlmann's process with some modifications. The acetylenic diol VIII was chlorinated with triphenylphosphin-carbon tetrachloride reagent under very mild condition in good yield. It was shown that the terminal iodo-ethynyl compound couples with the terminal acetylenes easier than any other halo-ethynyl compounds under Cadiot-Chodkiewicz condition. Therefore VII and X were coupled heterogeneously for a few hours at 0°C to give C₁₃-polyacetylenic dioxolane XIa with satisfactory yield. IR, UV and NMR spectra showed that the product consisted of four (E) and (Z) isomers in respect to the two double bonds. Catalytic hydrogenation of XIa over Adams platinum oxide gave a single product as indicated by GLC analysis. The GC-MS analysis also supported the integrity of the sole hydrogenation product as 2,2-dimethyl-4-undecanyl-1,3-dioxolane. As the separation of these four geometrical isomers was unsuccessful, after removal of the protecting group the resulting diol mixture was recrystallized from chloroform-hexane mixture to give the most easily crystallizable (S)-(E)-(E)-isomer Ia in pure state. The physical properties and UV and IR spectra of the recrystallized product were identical with those of the natural product and the racemate. In the NMR spectra the four olefinic protons appeared at δ=5.63 ppm (doublet, J=16 Hz), 5.90 (doublet, J=16 Hz), 6.38 (doublet, doublet, J=16, 3 Hz) and 6.41 (doublet, quartet, J=6, 7 Hz) and from their coupling constants, its (E)-structure was finally established. The ORD was measurable over the range of 600~400 nm. The (S) diol Ia exhibited a positive plain curve and its optical rotation at 589 nm ([α]₂⁰°C) was +18.4º. In view of the levorotation found for the natural product ([α]₁⁻⁰°C=−17º), it then follows that the natural safynol should have the antipodal (R)-configuration. Then, starting from (S)-2,3-O-isopropylidene-glyceraldehyde we undertook the synthesis of (R)-safynol by the same route as the preparation of (S)-safynol. Because of the troublesome preparation and NaIO₄-oxidation of 1,2-O-isopropylidene-d-sorbitol, the optical purity of the product was 38.8%, but it was satisfactory enough to assign the absolute configuration. The UV, IR and NMR spectra of synthetic (R)-safynol (Ib) were identical with those of (S)-safynol (Ia). The ORD curve of the (R)-safynol (Ib) showed an opposite trend to the (S) enantiomer and its optical rotation at 589 nm ([α]₂⁰°C) was −16.7º (Fig. 2). Therefore, the absolute configuration of the natural product was finally determined as (R). Although no elavorate data concerning the difference in optical properties of I isolated from sound and infected plants have been available, it seems unlikely that a plant could possibly be affected by infection to switch the steric course of biogenetic pathway so as to give the enantiomeric product, so that the phytoalexin, safynol, should also have the (R)-configuration.
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Antifungal activities of the synthetic products: In order to study the absolute configuration-biological activity relationship of safynol, antifungal assay was made with five typical pathogenic fungi; Ophiobolus miyabeanus Ito et Kuri, Fusarium sp. niveum, Rhizoctonia solani Kuhn, Phytophthora capsici and Phytophthora drechsleri. Each fungus was cultured at 25°C and 30°C on the potato-agar plate containing (R) and (S)-safynols in various concentrations. Inhibition of mycelial growth was measured after 2, 3 and 4 days and the median effective concentration (EC₅₀) of the optically active safynol was calculated by the probit method. EC₅₀ values and dose concentrations of perfect inhibition against the mycelial growth were summarized in Table I. EC₅₀ of (R)-safynol for five fungi varies from 10 to 35 ppm, and those of (S)-safynol from 6 to 70 ppm. These antifungal activities were in the same degree as those of the other phytoalexins. Among the tested fungi P. drechsleri is only a pathogen to safflower, however, great difference in activity of both (R) and (S) safynols has not been observed between P. drechsleri and other fungi. Therefore it can be claimed that the phytoalexin, safynol, has no selective antifungal activity to the pathogenic fungi and the absolute configuration has no bearing on the antifungal activity.

EXPERIMENTAL

Infrared spectra were recorded on a JASCO IRA-1 grating infrared spectrophotometer. ¹H-Nuclear magnetic resonance spectra were determined in CCl₄ or (CD₃)₂CO on a JEOL MH-60 (60 MHz) nuclear magnetic resonance spectrometer using TMS as an internal standard. Mass spectra were obtained at 75 eV utilizing a JEOL JMA-06 mass spectrometer with GC-MS system. ORD spectra were recorded on JEOL-ORD/UV-5.

(R)-(E and Z)-2, 2-Dimethyl-4-(4-trimethylsilyl-1-buten-3-ynyl)-1, 3-dioxolane (VIb)
The Wittig salt V (11.31 g, 0.026 mol) was suspended in dry THE (40 ml) under N₂ at -78°C and n-BuLi in hexane (20 ml, 0.026 mol) was added during 15 min. The mixture was kept at -20°C for 1 hr, and (R)-2, 3-O-isopropylidene-glyceraldehyde (3.35 g, 0.026 mol) in dry THE (4 ml) was slowly added to it. After 1 hr at -20°C and 1 hr at 0°C, 50 ml of Et₂O-hexane (1: 1) was added to the mixture, the solids were removed by filtration, and the filtrate was concentrated. The residue was separated by silica-gel column chromatography (eluted with Et₂O-hexane (19:1) and PLC (continuously eluted with Et₂O-hexane=1: 9) which showed two bands with RF 0.73 and 0.63 respectively (total yield 76.5%, Z: E=4: 3). (Z)-isomer: UV λₑₓₖ₅₉ nm (ε): 226.3 (10,000), 236.5 (14,200), 247 (12,100). IR cm⁻¹: 2160 (C=C), 1610 (CH=CH), 1385, 1375 (CMe₂), 1060 (CO), 850, 765 (SiMe₃).

<table>
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<th>Fungi</th>
<th>(R)-Safynol</th>
<th>(S)-Safynol</th>
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<tr>
<td>Ophiobolus miyabeanus Ito et Kuri</td>
<td>27.5 ppm</td>
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<td>Fusarium sp. niveum</td>
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<tr>
<td>Phytophthora drechsleri</td>
<td>19.4 ppm</td>
<td>14.5 ppm</td>
</tr>
<tr>
<td></td>
<td>&gt;50 ppm</td>
<td>108.5 ppm</td>
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TABLE I. INHIBITION OF MYCELIAL GROWTH OF FIVE FUNGI WITH (R) AND (S) SAFYNOL
NMR (10% in CCl₄): δ: 0.17 (9H, s, Me₂Si), 1.33 (6H, s, Me₂C), 3.47 (1H, d, d, J=7, 7 Hz, CHOCH₂O), 4.13 (1H, d, d, J=7, 7 Hz, CHOCH₂O), 4.97 (1H, m, CH₂O), 5.57 (1H, d, d, J=12 Hz, C=C-CH=CH), 5.97 (1H, d, d, J=12, 7 Hz, C=C-CH=CH). ORD (c=1.038 in EtOH) ν[R]₁₀₀₀ cm⁻¹: +1.04° (589 nm), +2.60° (500), +5.72° (450), +14.6° (400), +32.3° (350). MS m/e (%): 224 (M⁺, 4), 209 (23), 194 (34), 166 (44), 151 (100), 109 (38), 75 (39), 73 (42), 72 (39), 43 (18). (E)-isomer: ν[R]₁₀₀₀ cm⁻¹: 2180 (C=C), 1620 (CH=CH), 1385, 1375 (CMe₂), 1060 (CO), 960 (-CH=CH). NMR (10% in CCL₄) 6: 1.34 (6H, s, Me₂C), 3.10 (1H, d, d, J=7, 7 Hz, CHOCH₂O), 3.50 (1H, d, d, J=7, 7 Hz, CHOCH₂O), 4.93 (1H, m, CHOCH₂O), 5.67 (1H, d, d, J=12 Hz, CH=CH), 5.90 (1H, d, d, J=12, 7 Hz, CH=CH). (Z)-isomer: NMR (10% in CCl₄): δ: 1.34, 1.37 (each 3H, s, 3H, s), 3.50 (1H, d, d, J=7, 7 Hz, CHOCH₂O), 4.00 (1H, d, d, J=7, 7 Hz, CHOCH₂O), 4.27 (1H, m, CH₂O), 5.80 (1H, d, J=16 Hz, CH=CH), 6.10 (1H, d, d, J=16.7 Hz, CH=CH).  

(R)-(E and Z)-2, 2-Dimethyl-4-(4-iodo-1-buten-3-ynyl)-1, 3-dioxolane (VIIb) 

The separated Ag-salt was extracted with CH₂Cl₂ from the reaction mixture directly. Desilylation, iodination and purification procedures were the same as above. From 4.45 g of the trimethylsilyl dioxolane VI, 2.31 g of iodo dioxolane VII was obtained. IR ν[R]₁₀₀₀ cm⁻¹: 2180 (C=C), 1620 (CH=CH), 1390, 1380 (CH₂), 1060 (CO), 960 (E-CH=CH).  

(E)-1, 6-Dichloro-4-hepten-2-yne (IX) 

5-Hepten-2-yne-1, 4-diol (11.6 g, 0.1 mol) in DMF (25 ml) was mixed with Ph₃P (57.6 g, 0.22 mol) in CCl₄ (400 ml) in the dark under N₂ at room temperature. After stirring the mixture was concentrated and extracted with hexane. Hexane layers were washed with NaHCO₃-H₂O, dried, filtered, and concentrated, and the liquid residue was distilled to give VIII at 49~52°C under 0.14 mmHg (yield 70%). IR ν[R]₁₀₀₀ cm⁻¹: 2220 (C=C), 1625 (CH=CH), 950 (E-CH=CH). NMR (10% in CCl₄): δ: 1.57 (3H, d, J=7 Hz, CH₃), 4.13 (2H, m, CHOCH₂) 4.33 (1H, m, CH₂Cl), 4.74 (1H, m, CH₂O).  

(S)-(E and Z)-2, 2-Dimethyl-4-(1, 9-undecadiene-3, 5, 7-triynyl)-1, 3-dioxolane (VIIa) 

To the trimethylsilyl dioxolane VIa (3.35 g, 0.015 mol) in EtOH (100 ml) stirred at 20°C under N₂ was added dropwise AgNO₃ (5.1 g, 0.03 mol) dissolved in EtOH (100 ml) 1:1 over 30 min. After 30 min stirring the mixture was cooled to 0°C and H₂O was added dropwise AgNO₃. The mixture was stirred at 0°C for 2 hr, then KCN (0.7 g) in ice (20 g)-H₂O (10 ml) was added. After stirring was continued for 30 min, the precipitate was filtered off and organic solution was concentrated and extracted with CH₂Cl₂. The precipitate was dissolved in MeOH (5 ml) and added dropwise to a stirred solution of CuCl (30 mg), NH₂OH·HCl (200 mg) and EtNH₂ (40 % in H₂O, 8 ml) in the MeOH (8 ml) under N₂ at 0°C. Then iodo-dioxolane VIIa (2.4 g, 0.009 mol) in MeOH (5 ml) was added dropwise over 10 min. The mixture was stirred at 0°C for 2 hr, then KCN (0.7 g) in ice (20 g)-H₂O (10 ml) was added. Isolation by Et₂O extraction (3 x 40 ml) and silica gel column chromatography (Et₂O: hexane=1: 4) yielded the diene-triyne dioxolane. UV ν[R]₁₀₀₀ nm: 215, 224, 234, 246, 255, 270, 290, 308, 330, 354. IR ν[R]₁₀₀₀ cm⁻¹: 2220 (C=C), 1625 (CH=CH), 950 (E-CH=CH).  

(S)-2, 2-Dimethyl-4-(1, 5-undecadiene-3, 5, 7-triynyl)-1, 3-dioxolane (Xia) 

5-Heptene-1, 3-dyne X (0.81, 0.009 mol) prepared from dichloride IX by the known method was dissolved in MeOH (5 ml) and added dropwise to a stirred solution of CuCl (30 mg), NH₂OH·HCl (200 mg) and EtNH₂ (40 % in H₂O, 8 ml) in the MeOH (8 ml) under N₂ at 0°C. Then iodo-dioxolane VIIa (2.4 g, 0.009 mol) in MeOH (5 ml) was added dropwise over 10 min. The mixture was stirred at 0°C for 2 hr, then KCN (0.7 g) in ice (20 g)-H₂O (10 ml) was added. Isolation by Et₂O extraction (3 x 40 ml) and silica gel column chromatography (Et₂O: hexane=1: 4) yielded the diene-triyne dioxolane. UV ν[R]₁₀₀₀ nm: 215, 224, 234, 246, 255, 270, 290, 308, 330, 354. IR ν[R]₁₀₀₀ cm⁻¹: 2220, 2170 (C=C), 1620 (CH=CH), 1380, 1370 (Me₂C), 1050 (CO), 950 (E-CH=CH).

Octadehydro derivative 

Xia (60 mg) was shaken with platinum oxide (50 mg) in dioxane under hydrogen atmosphere for 1.5 hr. The filtrate was concentrated under reduced pressure. IR ν[R]₁₀₀₀ cm⁻¹: 1380, 1370 (CMc₂), 1060 (CO). NMR (10% in CCl₄): δ: 0.87 (3H, s, Me), 1.25 (26H, s, Me₆C⁺ (CH₂)₁₀), 3.3~4.0 (3H, m, CH₃C=O). GC-MS m/e (%): 256 (M⁺, 7), 241 (M⁺-CH₃), 125 (13), 111 (32), 101 (M⁺-(CH₂)₁₀CH₃), 26, 97 (58), 83 (84), 69 (84), 57 (59), 55 (51), 43 (CH₃C⁺=O, 100).
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(R)-2, 2-Dimethyl-4-(1, 9-undecadiene-3, 5, 7-triyynyl)-1, 3-dioxolane (XIb)

All experimental conditions were the same as above. From 0.66 g of 5-heptene-1, 3-diyne (X) and 2.0 g of iodo-dioxolane VIIb, 1.0 g of (R)-polyacetylenic dioxolane XIb was obtained. UV ăEtOHmax nm: 215, 224, 234, 246, 255, 270, 308, 330, 354. IR ƒËfilmmaxcm⁻¹: 2200, 2130 (C= C), 1620 (CH=CH), 1385, 1380 (CMe₂), 960 (E, CH=CH).

(S)-(E, E)-3, 11-Tridecadiene-5, 7, 9-triyynyl-1, 2-diol (Ia)

The diene-triyne dioxolane VIa (100 mg) was stirred for 8 hr in EtOH (20 ml)-HCl (2 N 3 ml) under N₂ at 20°C. The mixture was concentrated, then extracted with Et₂O (3 x 50 ml) and the combined extracts were washed with NaHCO₃-H₂O and dried, then concentrated. Crystallization of the residue from CHCl₃-hexane gave (S)-(E, E)-diol Ia in 25% yield. mp 123.5-124°C. Anal. Found: C, 77.37; H, 6.05. Calcd. for C₁₃H₁₂O₁: C, 77.97; H, 6.04%. UV ăEtOHmax nm (e): 214.5 (31,100), 225 (36,000), 234.8 (54,300), 245 (81,600), 254 (85,300), 268.5 (68,500), 289.5 (11,800), 308.5 (22,700), 329.7 (31,300), 353.7 (22,200). IR ƒËKBrmax cm⁻¹: 3250, 3200 (OH), 2190 (C= C), 1625 (CH=CH), 1070, 1040 (CO), 965, 950 (E, E CH=CH). NMR (10% in (CD₃)₂CO) 3: 1.83 (3H, d, d, J=7, 3 Hz, CH₃), 2.93 (1H, s, CH,OH), 3.52 (2H, d, J=5 Hz, CH,OH), 3.80 (1H, d, J=5 Hz, CHOH), 4.23 (1H, m, CHOH), 5.63 (1H, d, J=16 Hz, CH=CH-C=C), 5.90 (1H, d, J=16 Hz, C=C-CH=CH), 6.38 (1H, d, J=16 Hz, CH=CH-C=C). (R)-(E, E)-3, 11-Tridecadiene-5, 7, 9-triyynyl-1, 2-diol (Ib)

All experimental conditions were the same as above. From 0.5 g of the diene-triyne dioxolane XIb, 0.083 g of (R)-(E, E)-diol Ib was obtained. mp 122.5 - 123.5°C. Anal. Found: C, 77.99; H, 6.05. Calcd. for C₁₃H₁₂O₂: C, 77.97; H, 6.04. UV ăEtOHmax nm (e): 215 (31,900), 225 (35,800), 234.8 (51,200), 245 (71,600), 254 (75,200), 268.5 (60,300), 289.5 (10,300), 308.5 (18,700), 329.5 (26,000), 353.5 (18,400). IR ƒmaxcm⁻¹: 3220, 3180, (OH), 2190 (C≡C), 1620 (CH=CH), 960, 950 (E, E CH=CH). NMR (10% in (CD₃)₂CO) 3: 1.83 (3H, d, d, J=7, 3 Hz, CH₃), 2.92 (1H, s, CH,OH), 3.47 (2H, d, J=5 Hz, CH₂OH), 3.80 (1H, d, J=5 Hz, CHO), 6.37 (1H, d, J=16 Hz, CH=CH-C≡C), 6.40 (1H, d, J=7, 16 Hz, CH₃CH=CH=CH).

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REFERENCES