Short Communication

Isolation and Conformation of
(2*Z*,4*E*)-(+) -4'-Hydroxy- γ -ionylideneacetic Acid as a
Germination-suppressive
Substance from
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In our continuing search for new plant growth regulators from fungal metabolites,¹⁾ we found a germination-suppressive substance against lettuce seeds in an ethyl acetate extract of the culture broth of an unidentified species of *Stemphylium*, Hyphomycetes. The active principle has been isolated, and identified with an abscisic acid-related compound, (2*Z*,4*E*,1'*R*,4'*S*)-(+) -4'-hydroxy- γ -ionylideneacetic acid (I).²⁾ In addition, the unusual conformation of its cyclohexane ring has been revealed by high-field NMR analysis. This compound inhibited the germination of lettuce seeds (Great Lakes) at concentrations of more than *c.* 50 ppm³⁾ in the dark, whereas ($\pm$)-abscisic acid inhibited at concentrations of more than 5 ppm in a parallel test. This report is the first instance of a (2*Z*,4*E*)- γ -ionylideneacetic acid derivative being isolated as a germination-suppressive substance from the new fungal source.⁴⁾

The fungus identified as the genus *Stemphylium* was shake-cultured in a medium containing malt syrup, soybean flour, Pharmamedia and some other compounds, using Sakaguchi flasks in almost complete dark for 10 days. The culture filtrate (pH 8.4 and then adjusted to

pH 3) was partitioned against ethyl acetate, and column chromatography (BW-820MH silica gel, ethyl acetate-*n*-hexane) of the ethyl acetate extract gave the active fraction, after eluting with 40% ethyl acetate in *n*-hexane. This fraction was rechromatographed by low pressure LC (CQ-3 silica gel, chloroform-2-propanol (100:1)), and successively purified by preparative TLC (60 F₂₅₄s silica gel, chloroform-methanol (20:3)). The active substance obtained gave a violet spot on a silica gel TLC plate by spraying with a vanillin-sulfuric acid reagent. This active substance (*c.* 0.5 mg) was also isolated from a culture broth (1 l) of the fungus grown in a malt-sucrose medium. This germination-suppressive substance (GsS) was a colorless solid showing $[\alpha]_D^{20} +55^\circ$ (EtOH).

GsS exhibited the following spectral properties: EI-MS *m/z*: 250 (*M*⁺, very weak), 232, 217, 199, 187, 158, 147, 119, 111 (base peak) and 105; UV $\lambda_{\max}$ (MeOH) nm (ϵ): 260 (24000); IR $\nu_{\max}$ (CHCl₃) cm⁻¹: 3590 and 3400 (br, OH), 2800~2400 and 1682 (br, COOH), 1625 and 1598; NMR (Fig. 1) δ_H : 7.56 (Ha, br. d, 16^s), 6.36 (Hb, dd, 16, 9), 5.66 (Hc, br. s), 4.82 (Hd, 2H, br. s), 3.90 (He, dddd, 11, 11, 4, 4), 2.65 (Hf, br. d, 9), 2.51 (Hg, br. dd, 13, 4), 2.16 (Hh, br. dd, 13, 11), 2.03 (Me_A, br. s), 1.64 (Hi, br. dd, 13, 4), 1.49 (Hj, dd, 13, 11), 0.93 (Me_B, s) and 0.86 (Me_C, s). The IR spectrum of GsS showed both hydroxyl and carboxyl bands. It gave a *p*-bromophenacyl ester (mp 78~79°C; EI-MS *m/z*: 428 (*M*⁺ - 18) and 307 (⁷⁹Br-containing ions)) by treating with *p*-bromophenacyl bromide and *N*-ethyldiisopropylamine in acetonitrile, and yielded a methyl ester by treating with ethereal diazomethane; $[\alpha]_D^{20} +109^\circ$ (EtOH); EI-MS *m/z*: 264 (*M*⁺), 246, 231, 199, 187 and 125 (base peak); IR $\nu_{\max}$ (CHCl₃) cm⁻¹: 3450 (br), 1704, 1627 and 1599.

FIG. 1. ¹H-¹H COSY and ¹H-NMR Spectra of GsS (CDCl₃, 500 MHz).

The characteristic UV spectrum of GsS indicated the presence of an α , β , γ , δ -unsaturated acid chromophore, which was extended to a partial structure $-\text{CHf}-\text{CHb}=\text{CHa}-\text{C}(\text{Me}_\text{A})=\text{CHc}-\text{COOH}$ (Fig. 2) from the peculiar ^1H -NMR data of GsS.⁴⁾ This (2*Z*,4*E*)-3-methylpentadienoic acid moiety had the same side chain as that of the plant hormone, abscisic acid. The ^1H - ^1H COSY NMR spectrum of GsS (Fig. 1) clearly indicated the presence of another partial structure having a hydroxyl group $-\text{CHg}(\text{Hh})-\text{CHe}(\text{OH})-\text{CHi}(\text{Hj})-$. This spectrum further displayed some long-range couplings between Hd and Hh, Hj and Me_B, Hg and Hi, and Hf and Hi. The broad singlet of two protons at δ 4.82 (Hd) was assignable to a vinyl methylene group, and the remaining two methyl protons (Me_B and Me_C) to a *gem*-dimethyl group. These observations suggested that GsS was a 4'-hydroxyl derivative of (2*Z*,4*E*)- γ -ionylideneacetic acid, which was supported by ^{13}C -NMR data of the GsS methyl ester; δ (CDCl_3) 166.6, 151.0, 146.0 and 35.1 (C), 137.1, 129.0, 116.0, 68.0 and 58.0 (CH), 112.2, 44.2 and 41.2 (CH_2), and 50.1, 28.6, 28.0 and 21.0 (CH_3).²⁾

Vicinal coupling constants (J_e , $h=J_e$, $j=11\text{ Hz}$) and the presence of long-range couplings with a "W" 4 σ -bond arrangement (described above: Hg-Hi (1.5 Hz), Hf-Hi (*c.* 0.5 Hz)⁶⁾ and Hj-Me_B) indicated that the conformation of the cyclohexane ring in GsS was a chair form, and that the hydroxyl group on the cyclohexane ring was at the equatorial position. In addition, it was revealed that the pentadienoic acid side chain as the bulkiest substituent was unusual in being at the axial position. Therefore, the structure of GsS, excluding its absolute configuration, was assigned as that shown in **I**.

Oritani *et al.* have quite recently reported the isolation and structural determination of (2*Z*,4*E*)-4'-hydroxy- γ -ionylideneacetic acids as biosynthetic intermediates of fungal abscisic acid from *Cercospora cruenta*. One of these, (2*Z*,4*E*)-(+)-4'-hydroxy- γ -ionylideneacetic acid, had the same stereostructure as that of GsS.²⁾ Their physicochemical and spectral

FIG. 2. Partial Structures of GsS.

properties (those of GsS and its methyl ester have already been described, and ^1H -NMR data of the latter follow) were expectedly in good agreement with each other.^{2,7)} In their study to determine the configuration of the 4'-hydroxyl group, Oritani *et al.*, however, considered another chair form **II** with the equatorially oriented pentadienoate moiety, whose absolute configuration including the 1' position was determined by its chemical transformation to methyl (2*Z*,4*E*,1'*R*)-(+)-4'-oxo- α -ionylideneacetate.²⁾

The conformation of **I** was distinctly confirmed from NOE difference spectra of the GsS methyl ester in chloroform- d_1 . Irradiation of Hf (δ 2.65) clearly gave rise to differential signals on Ha (7.61), Hd (4.84, 2H, br. s) and Me_B (0.93), irradiation of Hd on Hf and Hg (2.50), irradiation of He (3.89) on Hg, Hi (1.62) and Me_B, irradiation of Me_B on He, Hf and Hi, and irradiation of Me_C (0.90) on Ha, Hb (6.32), Hf, Hi and Hj (1.48). These observations unequivocally indicated the presence of the axially oriented pentadienoic acid moiety at the 1' position, as well as the 1,3-diaxial relationship between He and Me_B in the chair form of that shown in **I**. This unusual conformation of **I** is attributable to both of a 1,3-diaxial repulsion between the 4'-hydroxyl and 2'-methyl groups, and a steric compression between one of the exocyclic methylene protons (Hd) and the pentadienoate moiety in **II**. One of the exocyclic methylene protons (Hd) possessed Hf in close proximity in **I**, whereas it closely possessed the pentadienoate moiety in **II**. Large differences of chemical shift between the exocyclic methylene protons ($\Delta 0.29 \sim 0.22$ ppm) have been observed in most 1'-nonsubstituted (2*Z*,4*E*)- γ -ionylidene-

acetic acids from *C. cruenta* except for **I**.^{2,7~9)} This was interpreted by a shielding effect of the equatorially oriented pentadienoate moiety on the neighboring one of their exocyclic methylene protons.

The unusual conformation of **I** is of interest in connection with its biological activity and biosynthetic relationship to abscisic acid.

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