Rapid Paper

Conformation and Metabolism of
(+)-(2*Z*,4*E*)-*trans*-1',4'-Dihydroxy-
γ-ionylideneacetic Acid, a Biosyn-
thetic Intermediate of (+)-Absciscic
Acid in *Cercospora cruenta*

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The stereochemistry of (+)-(2*Z*,4*E*)-*trans*-1',4'-dihydroxy- γ -ionylideneacetic acid, a major metabolite from *Cercospora cruenta*, a fungus found to produce (+)-abscisic acid, was reexamined as to its ^1H - ^1H -C α and Noesy 2D-NMR spectra, and it was proved to have a chair conformation with an axial pentadienoate moiety. Further, the metabolism of (+)-[^{14}C]-1',4'-dihydroxy- γ -ionylideneacetic acid in tomato plants suggested the possibility of it being a biosynthetic intermediate of ABA in plants.

In previous papers,¹⁻³⁾ we reported that *Cercospora cruenta*, a fungus producing (+)-abscisic acid (ABA, **4a**), a plant hormone, produced mainly (+)-(2*Z*,4*E*)-*trans*-1',4'-dihydroxy- γ -ionylideneacetic acid (**3a**) with

small amounts of (-)-*trans* and (+)-*cis*-(2*Z*,4*E*)-4'-hydroxy- γ -ionylideneacetic acids, (**1a**) and (**2a**), as biosynthetic intermediates of ABA. We proposed the chair conformations, (**2_B**) and (**3_B**),³ with an equatorially oriented 3-methyl-2,4-pentadienoate moiety for the corresponding methyl esters, (**2b**) and (**3b**), respectively.

Recently, Sassa *et al.*⁴⁾ isolated (+)-*cis*-4'-hydroxy- γ -ionylideneacetic acid (**2a**) as a germination-suppressive substance from the culture broth of *Stemphylium* sp. and clarified that **2a** had an uncommon chair conformation (**2_A**) with an axially oriented side-chain by examining the NOE difference in ¹H-NMR spectra of **2b** in chloroform-*d*₁. Their results prompted us to reexamine the conformation (**3_B**)³⁾ proposed on examination of the 1,3-diaxial interaction between 2'-methyl and 4'-hydroxyl groups in ¹H-NMR spectra. Also, it was necessary to reconfirm the stereochemistry of **3a** deduced from the previous results,^{2,3)} the biosynthetic pathway for (+)-ABA in *C. cruenta* proposed by us including the unusual inversion of the (4'*R*)-hydroxyl group of **1a** to (1'*S*,4'*S*)-1',4'-dihydroxy- γ -acid (**3a**). Further, we examined the metabolism of **3a** in tomato plants in order to investigate the possibility of it being a biosynthetic intermediate of ABA in plants.

(+)-1',4'-Dihydroxy- γ -acid (**3a**) was separated from the culture broth of *C. cruenta*

FIG. 1.

FIG. 2. ^1H - ^1H Cosy and Noesy 2D-NMR Spectra of (+)-(2Z,4E)-*trans*-1',4'-DiOH- γ -methyl ester (**3b**) (CDCl_3 , 400 MHz).

and then methylated with diazomethane to yield the pure methyl ester (**3b**), of which the carbon skeleton had already been established through its conversion to (+)-ABA methyl ester (**4b**).¹⁾ The conformation of **3b** was reexamined as to the NOE difference spectra in chloroform- d_1 . All protons of **3b** in the ^1H -NMR spectrum were assigned on ^1H - ^1H Cosy and Noesy 2D-NMR (Fig. 2). The ^1H - ^1H Cosy NMR spectra of **3b** indicated long-range couplings between H_h and H_e , and H_a and Me_A . Vicinal coupling constants ($J_{g,e} = J_{g,a} = 11 \text{ Hz}$) and the presence of long-range coupling with a "W" 4σ bond arrangement on H_f and H_c ($J = 2.5 \text{ Hz}$), and Me_B and H_a indicated that the conformation of **3b** had a

chair form (**3A**) with an equatorial hydroxyl group and an axial pentadienoate side-chain. The conformation (**3A**) was further confirmed by the NOE difference spectra of **3b** in chloroform- d_1 .

Irradiation (homo-gated decoupling) of H_g (δ : 3.97) caused clear differential signals on H_f (δ : 2.73), H_c (δ : 1.78) and Me_B (δ : 0.96); irradiation of H_k (δ : 6.61) on H_e (δ : 2.29), Me_c (δ : 2.02), H_a (δ : 1.59) and Me_A (δ : 0.98); irradiation of H_l (δ : 7.84) on H_d (δ : 1.87), H_i on H_h , H_h on H_f , Me_c on H_l , H_k , H_j ; $\text{Me}_{A,B}$ (δ : 0.97) on H_a , H_c , H_g , H_k and H_l . Irradiation (homo-spin decoupling) of H_g (δ : 3.97) confirmed the presence of couplings on H_a ($J_{a,g} = 11 \text{ Hz}$), H_c ($J_{c,g} = 4.6 \text{ Hz}$), H_e ($J_{g,e} = 11 \text{ Hz}$)

and H_f ($J_{g,f}=5.2$ Hz). Irradiation of H_h (δ : 5.0) showed long-range coupling on H_e ($J_{e,h}=1.5$ Hz), Me_c (δ : 2.02) on H_j . The above results showed that (+)-1',4'-dihydroxy- γ -acid (**3a**) had 1',4'-*trans* stereochemistry, as proposed in the previous paper,³⁾ and the same chair conformation (**3A**) with the axial side-chain as in the case of (+)-*cis*-4'-hydroxy- γ -acid (**2a**).⁴⁾ The easy dehydration of **2b** on treatment with *p*-toluenesulfonyl chloride in pyridine at 20°C and on MS-GLC analysis (200°C)³⁾ could be explained by the existence of another chair form (**2B**) with an axial hydroxyl group. The microbial conversion of (4'*R*)-4'-hydroxy- γ -acid (**1a**) into (4'*S*)-1',4'-dihydroxy- γ -acid (**3a**)²⁾ suggested the epimerization of the *sec*-hydroxyl group in *C. cruenta*.

The metabolism of (+)-[¹⁴C]-1',4'-dihydroxy- γ -acid (**3a**),²⁾ which had already been obtained by feeding (*R*)-[2-¹⁴C]-mevalonic acid into the culture of *C. cruenta*, in tomato plants was examined. The application of [¹⁴C]-**3a** (530×10^3 dpm) as an aqueous salt onto three-month-old cuttings of tomato plants was followed by incubation for 10 hr at room temperature. The plants were extracted with 80% methanol to yield an acidic fraction, which was methylated with diazomethane to give the methyl ester fraction (114×10^3 dpm). Separation of the ester fraction by TLC afforded the hot ABA methyl ester (**4b**) fraction (49×10^3 dpm), which was reduced with sodium borohydride to obtain *cis* and *trans* ABA diol methyl esters, (**5a**) and (**5b**)⁵⁾ (**5a**: 12.9×10^3 dpm, **5b**: 12.6×10^3 dpm). The calculated radioactivity of **4b** (25.5×10^3 dpm) in the latter, (**5a**) and (**5b**), indicated the 4.8% incorporation of **3a** into ABA.

Thus, the relatively high incorporation of **3a** into ABA in tomato plants, compared with those of other ionylideneacetic acids known as ABA analogs,⁶⁾ suggested the possibility of it being a biosynthetic intermediate of ABA in plants, although no γ -acids (**1a**, **2a** and **3a**) have been identified in plants yet. Also, this result suggested that the biological activity¹⁾ of **3a** might be due to its conversion into ABA in plants.

EXPERIMENTAL

¹H-NMR (400 MHz) spectra were recorded with a JEOL FT-NMR GX-400 spectrometer with TMS as an internal standard. Radioactivity was counted with a liquid scintillation spectrometer using a scintillator (toluene-MeOH-PPO-POPOP = 500 ml : 500 ml : 5.0 g : 0.1 g).

(+)-(2*Z*,4*E*)-Methyl *trans*-1',4'-dihydroxy- γ -ionylideneacetate (**3b**). From the culture broth of *C. cruenta* was separated (+)-1',4'-dihydroxy- γ -acid (**3a**), mp 206°C (from ethyl acetate), as described in the previous paper.¹⁾ **3a** was methylated with ethereal diazomethane and then purified by TLC on silica gel (Merck PF₂₅₄, benzene-ethyl acetate = 2 : 1) to give the pure methyl ester (**3b**) as a colorless oil. ¹NMR (CDCl₃) δ : 0.96 (Me_A , s), 0.98 (Me_B , s), 1.59 (H_a , dd, $J=13.2, 11$ Hz), 1.72 (H_b , s), 1.78 (H_c , ddd, $J=13.2, 4.6, 2.5$ Hz), 1.87 (H_d , s), 2.02 (Me_c , d, $J=1.5$ Hz), 2.29 (H_e , dddd, $J=13.2, 11, 1.5, 1.5$), 2.73 (H_f , ddd, $J=13.2, 5.2, 2.5$ Hz), 3.70 (Me_D , s), 3.97 (H_g , dddd, $J=11, 11, 5.2, 4.6$ Hz), 5.01 (H_h , t, $J=1.5$ Hz), 5.15 (H_i , t, $J=1.5$ Hz), 5.71 (H_j , m, $J=1.5, 0.5, 0.5$ Hz), 6.61 (H_k , dd, $J=16, 0.5$ Hz), 7.83 (H_l , dd, $J=16, 0.5$ Hz).

The metabolism of (+)-[¹⁴C]-1',4'-dihydroxy- γ -acid (**3a**) in tomato plants. The aqueous sodium salt of [¹⁴C]-**3a** (530×10^3 dpm) was applied to the cutting areas of tomato plants (three-month-old, Sugar-lamp, a dwarf variety of *Lycopersicon esculentum* Mill) (fresh weight, 280 g). The plants on paper were exposed to air until their weight decreased to 20%⁵⁾ and then they were stored in a plastic bag for 10 hr at room temperature. The plants were extracted with 80% MeOH, which contained 0.05% of 2,6-di-*tert*-butyl-*p*-cresol and ($\pm$)-ABA (2.0 mg) as a cold marker. The methanolic extract was concentrated and then adjusted to pH 2.5. The residual mixture was extracted with ethyl acetate. The organic layer was extracted with 2% aqueous NaHCO₃ to give neutral and acidic fractions. The acidic fraction was treated with ethereal diazomethane and then chromatographed on silica gel with ethyl acetate to give a methyl ester fraction (185 mg, 114×10^3 dpm). Separation of the ester fraction by preparative TLC on silica gel (benzene-ethyl acetate = 4 : 1) gave the hot ABA methyl ester (**4b**) fraction (R_f : 0.30 ~ 0.34, 49×10^3 dpm), which was reduced with NaBH₄ in 70% EtOH and then separated by TLC to give *cis* and *trans*-ABA diol methyl esters⁵⁾ (**5a**: 12.9×10^3 dpm) and (**5b**: 12.6×10^3 dpm).

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