Agric. Biol. Chem., **43** (7), 1613~1614, 1979

Short Communication

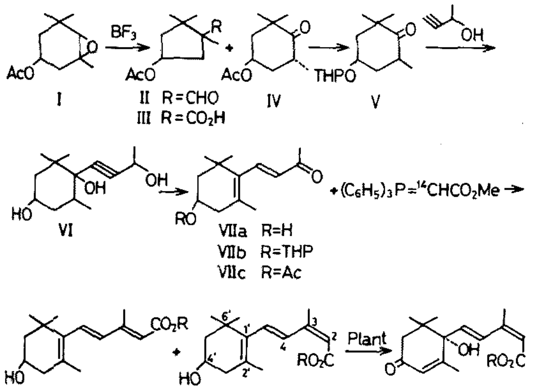
Synthesis and Metabolism of (±)-(2-^{14}C)-4′-Hydroxy-β-ionylideneacetic Acid

Takayuki Oritani and Kyohei Yamashita

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai

Received February 16, 1979

(±)-(2*Z*,4*E*)-4′-Hydroxy-β-ionylideneacetic acid (**IXa**) showed the growth inhibitory activity on rice seedlings[1] like that of abscisic acid (ABA, **Xa**), but there was no indication whether **IXa** was converted to ABA by plant or not. Therefore, the authors synthesized (2-^{14}C) labelled substrate (**VIIIa** and **IXa**) as shown in Scheme and examined the incorporation of these compounds into ABA by plant.



3,4-Epoxy-3,5,5-trimethylcyclohexyl acetate (I)[2] (40.0 g) was stirred with 20 ml of BF$_3$ etherate in 40 ml of benzene for 45 min to give 38.0 g of a mixture of the acetoxy–aldehyde (II) (10%) and the acetoxy–ketone (IV) (90%), bp131~139°C/16 mmHg (GLC analysis with 30% Silicone SE–30). After air was bubbled into the mixture, the mixture was washed with aqueous NaHCO$_3$ to give the *trans*-acetoxy-ketone (IV),[2] bp 131~134°C/17 mmHg. Acidification of the aqueous washing afforded the acetoxy acid (III) as an oil, NMR (CCl$_4$) δ: 0.92, 1.03, 1.07, 1.15, 1.21 (9H, probably a mixture of *trans* and *cis* isomer), 1.92 (3H, s), 5.03 (1H, br), 9.08 (1H, s). Alkaline hydrolysis of III gave the hydroxy acid,[3] mp 203~204°C. Alkaline hydrolysis of IV, followed by treatment with dihydropyran in the presence of *p*-toluenesulfonic acid, gave *cis*-4-tetrahydropyranyl (THP) oxy-2, 2, 6-trimethylcyclohexanone (V).[2,4] According to the method developed by Loeber *et al.*,[5] the ketone (V) was converted to 4′-hydroxy-β-ionone (**VIIa**) *via* the triol (VI). **VIIa**: UV λ_{max} (EtOH) nm: 292 and 222. **VIIa** (1.40 g) was stirred with 3.5 ml of dihydropyran in 30 ml of benzene in the presence of *p*-toluenesulfonic acid (20 mg) for 4 hr at room temperature. The product was chromatographed on alumina to give 4′-THPoxy-β-ionone (**VIIb**) as an oil (1.86 g, 95% yield), IR ν_{max}^{film} cm^{-1}: 1670, 1600, 1120, 1030, 970, 870. Also, acetylation of **VIIa** with acetic anhydride-pyridine afforded 4′-acetoxy-β-ionone (**VIIc**)[4] quantatively.

(2-^{14}C)-Bromoacetic acid (0.25 mCi, Code CFA-18, the Radiochemical Centre, Amersham) was methylated with diazomethane and then diluted with 2.5 g of the cold methyl bromoacetate. The (2-^{14}C)-bromoacetate was added to a solution of triphenylphosphine (5.0 g) in 50 ml of benzene. The precipitated phosphonium salt was treated with 2% sodium hydroxide to give (2-^{14}C)-methoxycarbonylmethylenetriphenylphosphorane (4.2 g). The ketone (**VIIb**, 1.0 g) was reacted with the (2-^{14}C)-phosphorane (500 mg) diluted with the cold phosphorane (1.0 g) in an oil bath at 145~150°C for 2 hr. The reaction mixture was chromatographed on alumina to give methyl 4′-THPoxy-β-inoylideneacetates as an oil (900 mg, 75.6% yield). Treatment of the latter with dilute ethanolic sulfuric acid gave methyl 4′-hydroxy-β-ionylideneacetates as a stereoisomeric mixture of (2*Z*)-isomer (**IXb**)[6] and (2*E*)-isomer (**VIIIb**)[6] (580 mg). IR ν_{max}^{film} cm^{-1}: 3380, 1715, 1605, 1230, 1150, 1070, 970. NMR (CCl$_4$) δ: 1.07 (6H, s), 1.72 (3H, s), 2.05 (s, (2*Z*)-CH$_3$ for **VIIIb**), 2.32 (s, (2*E*)-CH$_3$ for

IXb), 5.65 and 5.75 (1H), 6.00 (1H, d, $J=$ 15 Hz), 6.40 (d, $J=15$ Hz, (4*E*)-CH for **VIIIb**), 7.53 (d, $J=15$ Hz, (4*E*)-CH for **IXb**). The hydroxy–esters were consisted of 42% of **IXb** (t_R: 10.5 min) and 58% of **VIIIb** (t_R: 12.4 min) by GLC analysis with a column (1 m × 3 mm) of 10% Silicone SE–30 (column temp. 200°C, N_2 pressure; 0.62 kg/cm^2). GLC-MS: 264 (M^+) for **VIIIb** and 264 (M^+) for **IXb**. Alkaline hydrolysis of the esters (**VIIIb** and **IXb**) (350 mg) with 5% ethanolic sodium hydroxide gave (±)-(2-^{14}C)-4′-hydroxy-β-ionylideneacetic acid (a mixture of **VIIIa** and **IXa**) (312 mg, 1.57×10^4 dpm/mg). IR ν_{max}^{film} cm^{-1}: 3320, 3100 ~2560, 1680, 1600, 1240, 1170, 1040, 970, 880. Similarly, the reaction of **VIIc** with the phosphorane, followed by alkaline hydrolysis, afforded the hydroxy–acids (a mixture of **VIIIa** and **IXa**).* A solution of the (2-^{14}C)-acids (**VIIIa** and **IXa**) (85.0 mg, total 133.45×10^4 dpm, dissolved in 100 mg of $NaHCO_3$, 1 ml of EtOH, 30 ml of water) was applied to the shoots (250 g) of the three months old rice plants (Honen-wase, an ordinary variety of *Oryzae sativa*) *via* the cutting area. The plants were exposed to air on paper until 20~25% of their weight were lost.[7] After standing for 10 hr at room temperature, the plants were extracted with 80% methanol. By the usual manner, the evaporated residue of the extract was separated into the neutral, the acidic and the hydrophilic fractions. The acidic fraction (65.1 mg, total 43.68×10^4 dpm) was treated with diazomethane to give the methyl ester fraction, which was examined by TLC (Silica-gel, Merck H) with the solvent system of benzene–ethyl acetate (4: 1). Nine spots could be detected by spray of 5% H_2SO_4 followed by heating: 1; *Rf* 0.06~0.14, 2; *Rf* 0.18~0.21, 3; *Rf* 0.22~0.31 (main), 4; *Rf* 0.39 (small), 5; *Rf* 0.43~0.49, 6; *Rf* 0.55 (small), 7; *Rf* 0.59 (small), 8; *Rf* 0.69~0.71, 9; *Rf* 0.94~0.96. Spots 1, 4, 5, 6, 7, 8 and 9 were detected in the methyl ester fraction from a control sample, which was obtained from the rice plants (250 g). The third spot corresponded to the recovered substrate (**VIIIb** and **IXb**) (blue color). The second spot showed a strong green-yellow fluorescence[8] which was characteristic to methyl abscistate (**Xb**). The substance corresponding to the second spot was separated by preparative TLC (Silica-gel, Merck PF_{254}) to give the methyl abscisate (**Xb**) fraction (2.84 mg), 69% purity by GLC analysis(10% Silicone SE–30). Also, the methyl ester fraction from the control sample contained about 0.03 mg of **Xb** by GLC analysis. The CD curve of the **Xb** fraction in 0.005 N–H_2SO_4–MeOH showed a positive maximum at 262 nm and a negative maximum at 230 nm like those of (+)-natural ABA.[9] The **Xb** fraction was diluted with the cold methyl abscisate (**Xb**) and purified by preparative TLC twice to show the 2.2% incorporation (total 2.9×10^4 dpm). From the above result it was obvious that the substrate (a mixture of **VIIIa** and **IXa**) was converted into (+)-ABA by rice plant in 2.2% yield (or 5.2% if only one geometric isomer (**IXa**) was utilized). Also, it is suggested that the hydroxy–acid (**IXa**) or its related compound is on the biosynthetic pathway to ABA.

---

* As the separation of **VIIIa** and **IXa** by preparative TLC was unsuccessful, the mixture of **VIIIa** and **IXa** was used for the incorporation experiment in plant.

REFERENCES

1) Presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Nagoya, April 3, 1978. Abstracts of Papers p. 422.
2) T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **39**, 89 (1975).
3) R. D. G. Cooper, L. M. Jackman and B. C. L. Weedon, *Proceeding Chem. Soc.*, **1962**, 215.
4) K. Mori, *Tetrahedron Lett.*, **1973**, 723; *Agric. Biol. Chem.*, **37**, 2899 (1973).
5) D. E. Loeber, S. W. Russel, T. P. Toube and B. C. L. Weedon, *J. Chem. Soc.*, (C), **1971**, 404.
6) F. Kienzle, H. Mayer, R. E. Minder and H. Thommen, *Helv. Chim. Acta*, **61**, 2616 (1978).
7) B. V. Milborrow, *Biochem. J.*, **119**, 727 (1970).
8) R. Antoszewski and R. Rundnicki, *Anal.Biochem.*, **32**, 233 (1969).
9) B. V. Milborrow, *Planta*, **76**, 93 (1967).