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METABOLISM OF ABSCISIC ACID: BACTERIAL CONVERSION TO DEHYDROVOMIFOLIOL AND VOMIFOLIOL DEHYDROGENASE ACTIVITY

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Key Word Index—Abscisic acid; bacterial metabolism; dehydrovomifoliol; vomifoliol dehydrogenase; phytohormone.

Abstract—A species of *Corynebacterium*, capable of metabolizing abscisic acid (ABA), was isolated from soil. The organism converted ABA to dehydrovomifoliol [(±)-1′-hydroxy-4′-keto-α-ionone] as the major metabolite. A cell-free extract exhibited vomifoliol dehydrogenase activity. This suggests that vomifoliol is most likely the immediate precursor of dehydrovomifoliol.

INTRODUCTION

Abscisic acid (ABA) has been shown to be metabolized in plants either by oxidation to phaseic acid [1, 2] and dihydrophaseic acid [2, 3] or by conjugation to glucopyranosyl ABA [2, 4]. Also, it has been suggested that ABA could possibly be metabolized in other ways [5, 6].

Recently, phytopathogenic fungi such as *Cercospora rosicola* [7], *C. cruenta* [8] and *Botrytis cinerea* [9] have been shown to synthesize ABA. They provide a powerful tool for studies of ABA biosynthesis [8, 10, 11]. We recently surveyed microorganisms for ABA metabolism and isolated from soil a species of bacterium which metabolizes ABA. This paper reports the isolation and identification of dehydrovomifoliol as the major metabolite of ABA produced by the bacterium and the detection of vomifoliol dehydrogenase activity.

RESULTS AND DISCUSSION

A species of bacterium designated as *Corynebacterium* 433-3-2 was isolated from soil by enrichment with ABA as a single carbon source. The organism grew well on ABA in the presence of yeast extract and metabolized it into two major compounds. When ^{14}C-ABA (prepared by biosynthesis from acetate-2-^{14}C) was incubated with a 45-hr culture of the organism, the labeled ABA was rapidly metabolized (Table 1). After 1 hr of incubation, the substrate was completely digested and two major metabolites were produced. The levels of these metabolites remained constant thereafter and the ratio of activity of metabolite I to II was about 4:1.

When the organism was grown on 80 mg of ABA, 20.6 mg of metabolite I was isolated as a chromatographically pure compound. In a duplicate experiment 18.5 mg of metabolite I was isolated.

TLC analyses of the metabolite showed that it had the same mobility as an authentic sample of dehydrovomifoliol [(±)-1′-hydroxy-4′-keto-α-ionone] in three solvent systems. Also, HPLC analyses showed that it had identical retention times as authentic dehydrovomifoliol. Furthermore, the MS was identical to that of dehydrovomifoliol. MS *m/z*: (int. > 1% except $[M]^+$), 222 (0.6, $[M]^+$), 207 (1), 180 (3), 166 (19), 151 (3), 149 (6), 124 (100), 95 (8), 43 (37). These results showed that bacterium 433-3-2 metabolizes ABA and produces dehydrovomifoliol as the major metabolite.

Table 1. Relative activity of three radioactive compounds in growth medium of bacterium 433-3-2

	Incubation time (min)			
^{14}C compounds	0	3	60	120
ABA	100	65	0	0
Metabolite I	0	24	79	78
Metabolite II	0	11	21	22

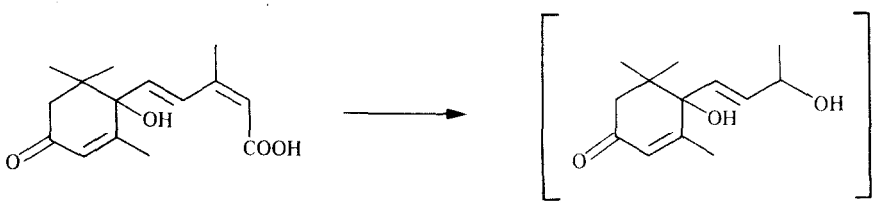
^{14}C-labeled ABA (6200 cpm/2 μg) was incubated with 0.2 ml of a 45-hr culture of bacterium 433-3-2. The incubation mixture (10 μl) was taken periodically for TLC analysis. Radiochromatograms were analysed with a Berthold LB 2382 Linear Analyzer; the relative activities were calculated from the peak integrals.

When 5 mg of vomifoliol was incubated with 5 mg of the enzyme preparation and NAD in 10 ml buffer, almost all of the vomifoliol was converted to dehydrovomifoliol after 18 hr of incubation at 23°. This showed that the cells possess vomifoliol dehydrogenase activity. Spectrophotometric analyses showed that the enzyme takes NAD as a cofactor, but not NADP. The presence of this enzyme suggests that vomifoliol is the immediate precursor of dehydrovomifoliol.

Vomifoliol is a naturally occurring compound structurally related to ABA. It has hormonal activity similar to that of ABA in potassium transport [12] and stomatal closure in epidermal strips [13]. This work suggests that the vomifoliol of plants may arise as a metabolite of ABA. The identity of metabolite II is currently being investigated.

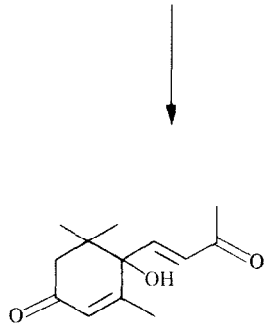
EXPERIMENTAL

Silica gel G plates were used for TLC. The plates were



Abscisic acid

Vomifoliol



Dehydrovomifoliol

developed with toluene–EtOAc–HOAc (100:50:3), EtOAc–hexane (3:2) and $CHCl_3$–EtOAc–HOAc (75:25:2). HPLC analyses were carried out on a Partisil PXS 5/25 ODS column (Whatman Inc.) by the procedure of Norman *et al.* [14]. GC/MS was performed using a 2 m × 2 mm ID glass column packed with 3% SP2250 on 100/120 Supelcoport coupled by a jet separator to VG7070 (70 eV, source 200°). GC was held at 100° for 2 min and programmed at 5°/min to 260°. Metabolite I was eluted at 19.75 min.

ABA was purchased from Burdick & Jackson Laboratories, Inc., Michigan. Dehydrovomifoliol was synthesized from α-ionone by the procedure of Roberts *et al.* [15]. Vomifoliol was prepared from dehydrovomifoliol by reducing it with $NaBH_4$ in MeOH at 0°and identified by GC/MS analysis. ^{14}C-ABA was prepared by incubating 28 μC of Na-acetate-2-^{14}C (2 μC/μM) with *C. rosicola* by a procedure similar to that of Bennett *et al.* [10]. After 24-hr incubation, the growth medium (10 ml) was acidified to pH 4 with 1 N HCl and passed through a C_{18} reversed-phase Sep-Pak cartridge (Waters Associates). The Sep-Pak was rinsed with 2 ml of H_2O and then eluted with 1.5 ml MeOH. The eluate was concd to 0.2 ml, diluted with 1.5 ml of H_2O, acidified to pH 3 with 1 N HCl and extracted with three 0.5-ml portions of EtOAc. The extracts were combined, washed with 0.5 ml H_2O and evaporated in vacuum. The residue was taken up in 0.5 ml CH_2Cl_2 and chromatographed on a 2.0 ml column of Sepralyte diol (Analytichem) packed in CH_2Cl_2. The column eluate was passed through a Berthold LB 503 HPLC radioactivity monitor, containing a siliconized glass scintillator cell. The column was first eluted with a linear gradient formed from 10 ml of CH_2Cl_2 and 10 ml of CH_2Cl_2–Et_2O (98:2) and then with a linear gradient formed from 10 ml of CH_2Cl_2–Et_2O (98:2) and 10 ml of CH_2Cl_2–Et_2O (95:5). The ABA was eluted in the first 12 ml of the second gradient. Evaporation of the solvent gave 320000 cpm of ABA, radiochemically pure by TLC.

Bacterial growth. The growth substrate, 40 ml of a mineral salt medium [16], 80 mg ABA and 40 mg yeast extract, was placed in a 125 ml flask, inoculated with *Corynebacterium* 433-3-2 and incubated on a shaker at 23°. After 48-hr incubation, cells were collected by centrifugation, washed with 0.1 M KPi buffer at pH 7 and frozen until used for enzyme analysis. The supernatant was used for metabolite analyses.

Isolation of metabolite I. The growth medium was worked up using a C_{18} reversed-phase Sep-Pak by the procedure of Bennett *et al.* [10] and resulted in the isolation of metabolite I as a chromatographically pure compound.

Preparation of enzyme. All operations were carried out in an ice bath. Frozen cells (1 g) were suspended in 50 ml of 0.1 M KPi buffer at pH 7 containing 10^{-3} M dithiothreitol and disrupted in a Rosset flask with a Branson sonifier, J-22. The supernatant from centrifugation at 20000 *g* for 10 min was brought to 0.9 saturation with $(NH_4)_2SO_4$. The mixture was centrifuged at 20000 *g* for 15 min and the ppt dissolved in a minimal portion of 0.01 M KPi buffer at pH 7 and used for enzyme analyses.

Assay method. Vomifoliol dehydrogenase was assayed by following the increase in optical density at 340 nm due to the formation of NADH. Activity was assayed in 1 ml of a reaction mixture containing 0.1 M Tris buffer at pH 8.5, 5×10^{-4} M NAD, 1×10^{-3} M vomifoliol and an appropriate amount of enzyme. Changes in absorbance were measured at 23° in a standard silica cuvette of 1-cm light path. The metabolite of vomifoliol produced by the enzyme preparation has R_fs identical to those of authentic dehydrovomifoliol by TLC with three solvent systems.

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