Occurrence of Jasmonic Acid in the Red Alga Gelidium latifolium

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The growth regulators (−)-jasmonic acid (JA) and its 7-isomer were identified by GC-MS in the red alga Gelidium latifolium. The ratio of JA:7-iso-JA was approximately 93:7. The endogenous level amounted to 0.7 μg/0.4050g fresh weight.

Introduction

The plant growth regulator (−)-jasmonic acid (JA), isolated as a plant growth inhibitor from the pericarp of Vicia faba [1], and its methyl ester, a potent senescence promoter from Artemisia absinthium [2], are widely distributed within higher plants [3]. However, there are very few reports of its occurrence in lower plants. Aldridge et al. [4] described JA as a product of the fungus Lasiodiplodia theobromae (Pat.) Griff. et Maubl. and Miersch et al. [5] isolated from the same fungal species (syonym Botryodiplodia theobromae (Pat.) its isomer (−)-7-iso-jasmonic acid (7-iso-JA). The green alga Chlorella pyrenoidosa (strain 211/8b) possesses the complete enzyme system for JA biosynthesis, but JA could not be found as native compound in this organism [6].

Recently, Ueda et al. [7] isolated minute quantities of JA (1.5 ng/g dry weight) from Euglena gracilis Z., an eukaryotic flagellate (Chlorophyta). Minute levels of JA and JAME were also detected in a Chlorella sp. and JA was found in the prokaryotic Spirulina maxima (Cyanophyceae), too [7, 8]. Furthermore, JA and related compounds were also identified in several Equisetum species (Pteridophyta) [9].

Within our research programme on the physiology of macrophytic algae [10, 11] we have begun to investigate the endogenous levels of plant growth regulators, including JA and related compounds. Here we describe the identification of JA and its 7-isomer in the red alga Gelidium latifolium.

Materials and Methods

The branched red alga Gelidium latifolium Born. (Rhodophyta) was collected from the Black Sea (Bay of Sewastopol, Krim peninsula) to a depth of 50 cm. The algae (length: 3–5 cm, 45 g fresh weight) were frozen, homogenized with MeOH using a blender, filtered and the remaining tissue extracted twice with 80% MeOH. The combined MeOH extracts were evaporated to aqueous, acidified to pH 3.0, partitioned with EtOAc and evaporated to dryness. This extract was subsequently chromatographed on a column of DEAE-Sephadex A-25 (1.1 x 45 cm; [12]). The fractions eluted with 0.25 and 0.5 M HAc in 80% MeOH were monitored by TLC (¼ of each fraction, silica gel GF254, CHCl3; EtOAc:acetone:HAc = 40:10:5:1). The JA-containing fractions were combined, methylated with ethereal diazomethane, purified on Adsorbex RP 18 (40 μm, 400 mg, Merck) with an increasing gradient of MeOH in water (5% steps, from 40% MeOH) and again monitored by TLC (hexane: EtOAc:HAc = 60:40:1). The JA fractions (60–65% MeOH) were evaporated to dryness and analyzed by GC and GC-MS applying the following conditions: GC – steel column (2 m × 4 mm), Supelcoport (100–120 mesh) coated with OV 225 (3%), carrier gas: N2, 45 ml/min, column temperature: 180 °C, GC-MS – steel column (1.5 m × 2 mm), Gaschrom Q (100–120 mesh) coated with OV 225 (3%), carrier gas: He, 15 ml/min, column temperature: 180 °C; 70 eV.

For GC the sample was dissolved in 10 μl benzene and 1/8 injected (8 replicates), for GC-MS 1/8 was injected. The remaining sample was reduced by NaBH4 [13] and again analyzed by GC (conditions as above, except: column temperature – 190 °C, N2 – 35 ml/min) to determine the ratio between JA and its 7-stereoisomer (JAME is reduced...
to 6-epi-7-iso-CAMe ($R_t = 7.8$ min) and 7-iso-CAMe ($R_t = 7.8$ min), 7-iso-JAME is reduced to 6-epi-CAME ($R_t = 9.3$ min) and CAMe ($R_t = 10.2$ min); the structural data and natural occurrence of which were recently reported [14]).

Results and Discussion

After chromatography of the EtOAc extract on DEAE-Sephadex A-25 we detected in fractions eluted with 0.25 m HOAc a JA like spot by monitoring on TLC ($R_f = 0.43$). After methylation, purification on Adsorbex RP 18 and TLC ($R_f$) we detected JAME in fractions eluted with 60–65% MeOH. In GC two peaks occurred, the first one ($R_t = 9.3$ min) corresponded to authentic JAME (32 $\mu$g; 0.7 $\mu$g/g fresh weight) and the second peak ($R_t = 11.1$ min) to authentic 7-iso-JAME (1.85 $\mu$g; 0.04 $\mu$g/g fresh weight). The ratio between JAME and 7-iso-JAME amounted to 94.3:5.7. The identity with JAME [1] and 7-iso-JAME [5] was proved by GC-MS ($R_{7iso-JAME} = 4.0$ min, $R_{JAME} = 4.6$ min). MS (JAME) $m/z$ (rel. int.): 224 (M+, 30), 206 (6), 193 (14), 156 (24), 151 (36), 135 (17), 109 (19) and 83 (100). MS (7-iso-JAME) $m/z$ (rel. int.): 224 (M+, 17), 206 (8), 193 (7), 156 (16), 151 (29), 135 (11), 109 (23) and 83 (100).

The ratio between JAME and 7-iso-JAME determined from the NaBH$_4$-reduced sample, in order to avoid isomerization of 7-iso-JAME to JAME during GC [15], was 93.4:6.6 (6-epi-7-iso-CAME + 7-iso-CAME and 6-epi-CAME + CAMe) and corresponded very well to the GC determination of JAME and 7-iso-JAME. The ratio of JA: 7-iso-JA in young fruits of Vicia faba was determined to be about 65:35 [13], and in black and green tea between 30:70 and 70:30 depending on the tea type [15]. A fungal strain of Botryodiplodia theobromae isolated from orange fruits forms exclusively 7-iso-JA [5]. The biosynthetic pathway of JA in plants should logically yield 7-iso-JA [16, 17]. Isomerization to the JA configuration may take place after any of the β-oxidation steps following the formation of phytodienic acid. In comparison to JA the 7-iso-JA is the more active compound in bioassays [18–20], and its methyl ester seems to be the essential odoriferous agent [21]. Possibly, only 7-iso-JA is the important biologically active compound, and it is therefore necessary to quantify it simultaneously to JA.

The occurrence of JA in Gelidium latifolium, Euglena gracilis Z., Chlorella sp. and in Spirulina maxima [7, 8] indicates that in addition to being present in higher plants [3] it is also distributed in lower plants, or at least these lower plants are capable of forming JA [6].

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