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 JA/g 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 promotor 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 (synonym *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 algal 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 began 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 × 45 cm; [12]). The fractions eluted with 0.25 and 0.5 M HOAc in 80% MeOH were monitored by TLC (1/5 of each fraction, silica gel GF_{254}, $CHCl_3$:EtOAc:acetone:HOAc = 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:HOAc = 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: N_2 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 1/20 injected (8 replicates), for GC-MS 1/10 was injected. The remaining sample was reduced by $NaBH_4$ [13] and again analyzed by GC (conditions as above, except: column temperature – 190 °C, N_2 – 35 ml/min) to determine the ratio between JA and its 7-stereoisomer (JAMe is reduced

Abbreviations: JA, (−)-jasmonic acid; 7-iso-JA, (+)-7-iso-jasmonic acid; CA, (+)-cucurbic acid; 6-epi-CA, (+)-6-epi-cucurbic acid; 7-iso-CA, (+)-7-iso-cucurbic acid; 6-epi-7-iso-CA, (+)-6-epi-7-iso-cucurbic acid; Me, methyl ester of the corresponding compound.

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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 (⅕) 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 µg; 0.7 µg/g fresh weight) and the second peak (R_t = 11.1 min) to authentic 7-iso-JAMe (1.85 µg; 0.04 µg/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_{tJAMe} = 4.0 min, $R_{t7\text{-iso-JAMe}}$ = 4.6 min). MS (JAMe) *m/z* (rel. int.): 224 (M^+, 30), 206 (6), 193 (14), 156 (24), 151 (36), 135 (17), 109 (22) 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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