Pergamon

Phytochemistry, Vol. 47, No. 3, pp. 327–329, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

PII: S0031-9422(97)00617-1

A JASMONIC ACID CONJUGATE, *N*-[(−)-JASMONOYL]-TYRAMINE, FROM *PETUNIA* POLLEN

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(*Received in revised form* 23 *June* 1997)

Key Word Index—*Petunia hybrida*; Solanaceae; pollen; *N*-[(−)-jasmonoyl]-tyramine; (−)-jasmonic acid.

Abstract—A new jasmonate, *N*-[(−)-jasmonoyl]-tyramine, was identified from petunia pollen in which (−)-jasmonic acid was detected and quantified.

INTRODUCTION

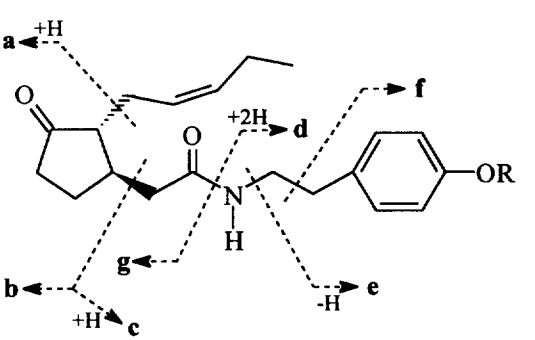
Jasmonic acid (JA) and structurally related compounds (jasmonates) are widely distributed in the plant kingdom. Exogenously applied to plants jasmonates induce a large number of physiological effects. As endogenous, biosynthetically formed compounds they are involved in the signal transduction between certain induced stress phenomena and the expression of specific genes as a response (reviews [1, 2]). Flowers are good sources for jasmonates such as their volatile methyl esters or amino acid conjugates [2–5]. JA and its methyl ester have been isolated from pollen and anthers of *Camellia* species. Studying their physiological effects only JA, but not its methyl ester, inhibited pollen germination [6]. An *Arabidopsis* mutant lacking unsaturated fatty acids and as a consequence lacking jasmonate shows male sterility [7]. Isoleucine conjugates of both JA and iso-cucurbic acid were isolated recently from mature *Pinus mugo* pollen, whereas the free acids could not be detected [8]. These findings implicate a possible role of jasmonates in the maturation and/or germination processes of pollen.

In preliminary experiments using an immunoassay for JA and its amino acid conjugates we noted the occurrence of an unknown neutral jasmonate in extracts of petunia pollen.

The present paper describes, for the first time in plants, the isolation and structural elucidation of *N*-[(−)-jasmonoyl]-tyramine (**1**) and the detection of (−)-JA in *Petunia* pollen extracts.

RESULTS AND DISCUSSION

Pollen of *Petunia hybrida* was extracted with ethanol and aqeous methanol followed by separation and purification of (−)JA and *N*-[(−)-jasmonoyl]-tyramine (**1**) (Fig. 1) by chromatographic methods (Extrelut, DEAE-Sephadex A25, TLC, C_{18}-cartridges, HPLC). Structural elucidation of **1** was achieved by LC-MS-MS, GC-MS, CD and immunological reactions. During the isolation steps an immunoassay based on polyclonal antibodies for methyl (−)-jasmonate [9] could be used for the detection of both compounds. In ion exchange chromatography compound **1** eluted with 0.25% acetic acid, typical for weak acidic substances without carboxylic acid groups, e.g. phenolic compounds, whereas JA eluted in fractions typical for mono carboxylic acids [10]. The GC mass spectral R_t and mass spectrum of JA methyl ester were identical with those of authentic samples [11]. The stereochemical assignment is supported by the immunological specifity of the antibodies which are specific for (−)-JA and do not show cross-reactivity for (+)-JA. Using (D_6)JA as internal standard for GC-MS [12] JA was quantified to 623 ng in 1 g pollen.



R = H **1**

R = Ac **2**

Fig. 1. Mass spectral fragmentation of *N*-[(−)-jasmonoyl]-tyramine (**1**) and its acetate (**2**) under EI and ES conditions.

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The positive ion electrospray (ES) mass spectrum of **1** showed a $[M+H]^+$ peak at *m/z* 330. The CID spectrum of *m/z* 330 displaying prominent ions at *m/z* 151 (**b**, jasmonoyl moiety) and 138 (**d**, tyramine moiety) was in good agreement with that of authentic *N*-[(−)-jasmonoyl]-tyramine (Fig. 1). The key ion at *m/z* 121 originated by loss of NH_3 from ion **d**. In accordance to the CID spectra of *N*-[(−)-jasmonoyl]-amino acid conjugates under ES conditions, ions at *m/z* 133, 109 and 93 are also indicative of a jasmonic acid moiety [13].

Compound **1** was acetylated to yield compound **2**, which was investigated by GC mass spectrometry. Both the R_t and the EI mass spectrum of isolated and authentic **2** were in good agreement. Besides the $[M]^+$ at *m/z* 371 key ions of type **a**–**g** appear (Fig. 1). The mass spectrum of **2** is very similar to that of methyl esters of *N*-[(−)-jasmonoyl]-amino acid conjugates [14]. The CD curves of isolated and authentic **1** are identical, the negative Cotton-effect directs to a (−)-jasmonoyl moiety in compound **1** and is due to the absolute configuration (1*R*,2*R*) presented in Fig. 1 [15]. Furthermore, the immunoreactivity of **1** also points to (−)-structures because of missing cross-reactivity of (+)-jasmonoyl-compounds [9].

Compound **1** is the first example of a conjugate of (−)-JA with an amine in plants. In addition, (−)-JA was described for the first time in *petunia* pollen. Compound **1** can be discussed as a metabolite of *N*-[(−)-jasmonoyl]-(*S*)-tyrosine which we could not detect in *petunia* pollen. However, the tyrosine conjugate was isolated from *Vicia faba* flowers as one of three JA conjugates containing aromatic amino acids [5, 14].

EXPERIMENTAL

Chromatographic methods. Prep. TLC: aluminium sheets silica gel 60 with concentrating zone (Merck), $CHCl_3$–EtOAc–HOAc (14:6:1), detection by anisaldehyde reagent and heating for 5 min at 120° [16] or immunoassay with substances recovered from gel zones with MeCN; R_f-value: JA 0.49, **1** 0.28. HPLC: Eurospher 100-C18, (5 μm, anal.: 250 × 4 mm, prep.: 250 × 10 mm), elution with MeOH–0.2% HOAc in H_2O (3:2), flow rate 1 ml min^{-1} (anal.) 3 ml min^{-1} (prep.), UV detector 210 nm, R_t (min) anal.: JA 5.5, **1** 5.5, prep. **1** 9.0; LC-MS-MS: the positive ion electrospray (ES) and the LC-MS-MS measurements were obtained from a Finnigan TSQ 7000 instrument (electrospray voltage 5 kV, sheath gas and auxillary gas N_2) combined with a constMetric 4100 HPLC instrument equipped with a LiChrospher 100 RP18-column (5 μm, 2 × 100 mm), elution with MeCN–H_2O (containing 0.2% HOAc), 13:7, flow rate 0.2 ml min^{-1}; R_t (min) **1** 2.0. The CIDMS during the HPLC run: collision energy −25 eV; collision gas, Ar; collision pressure 226.6×10^{-3} Pa. GC-MS (MD-800, Fisons Instruments): 70 eV EI, source temp. 200°, column DB-1 (J&W, 30 m × 0.25 mm, 0.25 μm film thickness), inj. temp. 250°, interface temp. 300°; He 1 ml min^{-1}; splitless injection; column temp. programme: 1 min 170°, 25° min^{-1} to 270°, 20 min 270°; R_t-values of **1** 2958 (isolated), 2962 (authentic); column DB5MS (15 m × 0.32 mm, 0.25 μm film thickness), He 1.3 ml min^{-1}, temp. programme: 1 min 60°, 25° min^{-1} to 110°, 10° min^{-1}, 25° min^{-1} to 290°, R_t (min): JA 8.17.

Plant material. Plants of *Petunia hybrida* HORT cv. 'Festival' were grown in a greenhouse and pollen collected from March to September in 1996. The mixt. of pollen and anthers was air-dried for 3 days, then suspended into petrol and sepd by filtration. After a second filtration pollen was suspended in dry EtOH (1 g in 5 ml) and stored at −20°.

Extraction and isolation. Pollen (20.74 g dry wt in 100 ml EtOH) was homogenized by an Ultra-Turrax for 10 min, sonicated for 5 min and filtered. Extraction of the remainder was repeated × 3 with 100 ml 80% MeOH. Combined extracts were evapd to the aq. phase which was kept at −20° for 24 hr and then centrifuged. The supernatant was evapd to 25 ml and acidified with 1 N HCl to pH 3 and loaded onto a column (3 cm × 30 cm) filled with Extrelut (Merck). Jasmonates could be eluted with 300 ml EtOAc, dried with dry Na_2SO_4 and evapd (residue 195 mg). Further purification was done on DEAE-Sephadex A25 (Ac, 50 ml) with a gradient of HOAc in 80% MeOH according to [10]. Aliquots (1/100) of frs were tested by an enzyme linked immunoassay [9].

Immunoreactive frs eluted with 0.25 N HOAc during DEAE-Sephadex A25 sepn were evapd. 9/10 of the fr. were purified directly, 1/10 after addition of 100 ng (D_6)JA for quantification [12], on Li-Chrolut RP-18 (Merck) with a gradient of MeOH containing 0.2% HOAc in H_2O. Frs eluted between 50–60% MeOH were evapd and chromatographed on anal. HPLC. The fr. at R_t = 5.0–6.0 was evapd and methylated with ethereal CH_2N_2 for 10 min and analysed by GC-MS.

(−)-*Jasmonic acid* (1*R*,2*R*)-3-oxo-2-(2*Z*-pentenyl) cyclopentane-1-acetic acid. 623 ng g^{-1} pollen. EIMS (70 eV) identical to that of authentic JA [11]. Immunoreactive frs eluted with 0.25% HOAc were purified by prep. HPLC and prep. TLC using the immunoassay for monitoring jasmonates. Further prep. HPLC gave 540 μg of **1**.

N-[(−)-*Jasmonoyl*]-*tyramine* *N*-[(1*R*,2*R*)-3-oxo-2-(2*Z*-pentenyl) cyclopentane-1-acetyl]-2-(4-hydroxyphenyl)ethyl amine (**1**). Positive ion ESCIDMS: *m/z* (rel. int.) of **1**: 193 **g** (6), 167 (27), 151 **b** (98), 138 **d** (17), 133 (21), 121 [**d**-NH_3]$^+$ (100), 109 (12), 93 (30), (see Fig. 1). CD of **1**: synthetic: $\Delta_{297.4} = -3.00$ (MeOH, 1.25×10^{-2} M); endogenous: $\Delta_{298.4} = -2.95$ (MeOH, 1.05×10^{-3} M); identical with synthetic **1** prepd from (±)-JA and 2-(4-hydroxyphenyl)ethyl amine (tyramine) according to ref. [17] and the resulting enantiomers were sepd into (−)-**1** and (+)-**1** according to ref. [18].

Compound **1** was acetylated by a mixt. (100 μl) of Ac_2O–pyridine (1:2) at 20° for 24 hr. Solvents were

evapd, 1 ml 1 N HCl added and **2** recovered by extraction with $CHCl_3$ yielding.

N-[(−)-*Jasmonoyl*]-O-*acetyl-tyramine N*-[(1*R*,2*R*)-3-oxo-2-(2*Z*-pentenyl) cyclopentane-1-acetyl]-2-(4-acetoxyphenyl)ethyl amine (**2**). EIMS of **2** (70 eV) *m/z* (rel. int.): 371 $[M]^+$ (5), 353 (4), 303 **a** (2), 221 **c** (13), 193 **g** (4), 180 **d** (3), 162 **e** (26), 151 **b** (5), 120 [**e**-$CH_2CO]^+$ (100), 107 [**f**-$CH_2CO]^+$ (17), 72 [**c**-$CH_2C_6H_4OAc]^+$ (14), (see Fig. 1) (identical to the EIMS of synthetic *N*-[(−)-jasmonoyl]-*O*-acetyl-tyramine).

Enzyme immunoassay. JA and **1** were detected by an enzyme immunoassay using polyclonal antibodies [9]. Samples were methylated prior to immunoassay with ethereal CH_2N_2 for 10 min.

Acknowledgements—The authors are indebted to Mrs E. Schneider for technical assistance, Mrs C. Gebhardt for immunoassays, Mrs C. Kuhnt for GC-MS analysis and Mrs M. Süße for CD measurement.

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