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SHORT REPORTS

N-[(−)-JASMONOYL]-*S*-TRYPTOPHAN AND A RELATED TRYPTOPHAN CONJUGATE FROM *VICIA FABA*

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Key Word Index—*Vicia faba*; Fabaceae; jasmonic acid; conjugates; *N*-[(−)-jasmonoyl]-*S*-tryptophan.

Abstract—*N*-[(−)-Jasmonoyl]-*S*-tryptophan has been isolated from flowers of *Vicia faba* and its structure elucidated by spectroscopic methods. An additionally isolated minor conjugate might be identical with *N*-[(+)-cucurbinoyl]-*S*-tryptophan

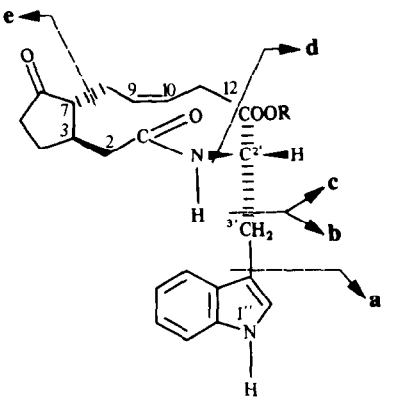
INTRODUCTION

Conjugates are widespread metabolites of plant hormones and other plant growth regulators possessing physiological significance [1–5]. Recently, we found *N*-[(−)-jasmonoyl]-*S*-tyrosine [6] to be a native amino acid conjugate of the endogenous plant growth regulator (−)-jasmonic acid [(−)-JA] in flowers of *Vicia faba*. In this paper, we present data on the occurrence of a further amino acid conjugate of (−)-JA and an analogue in broad bean flowers. These investigations were based on a radioimmunoassay (RIA) for (−)-JA [Knöfel, H.-D. *et al.*, unpublished] and various chromatographic techniques. This combination has proved to be a useful tool for detecting such amino acid conjugates exhibiting high immunoreactivity.

RESULTS

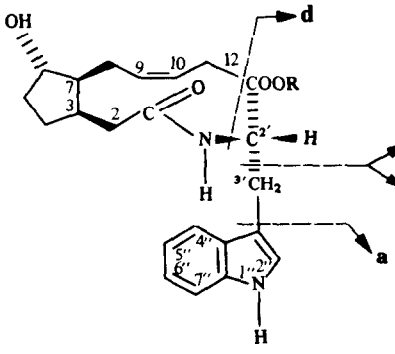
Extracts from flowers of *Vicia faba* yielded in addition to *N*-[(−)-jasmonoyl]-*S*-tyrosine [6] a less polar immunoreactive fraction IRF (isolation procedure according to [6], see Experimental). The methylated IRF was separated into fractions IRF-1-Me and IRF-2-Me, which have been analysed by MS, ^{1}H NMR and ORD. The positive ion mass spectra of IRF-1-Me ($[M]^+$ at *m/z* 410) and IRF-2-Me ($[M]^+$ at *m/z* 412) showed key fragments at *m/z* 117 $[a+H]^+$, *m/z* 130 $[b]^+$ and *m/z* 201 $[d-H]^+$ which reveal the presence of a tryptophan moiety in both compounds. The presence of a *c*-type ion at *m/z* 279 in the negative ion mass spectrum of IRF-1-Me ($[M-1]^-$ at *m/z* 409) gave evidence for an unchanged jasmonic acid residue in the molecule. From these data as well as those of ^{1}H NMR and ORD spectroscopy of IRF-1-Me the structure of the isolated IRF-1 was established to be *N*-[(−)-jasmonoyl]-*S*-tryptophan (**1**).

The negative ion mass spectrum of IRF-2-Me ($[M-1]^-$ at *m/z* 411) exhibits a *c*-type ion at *m/z* 281 ($[c-H]^-$) indicating that its tryptophan linked acid moiety contains 2 more hydrogen atoms than that of **2**. In the ^{1}H NMR spectrum of IRF-2-Me the signal pattern and the position of the two olefinic protons (δ5.32–5.49) indicate the intact 9, 10 double bond, whereas a new signal appears at δ3.85 (1H, *m*), the typical chemical shift range



1 R = H

2 R = Me



3 R = H

4 R = Me

of a methin proton attached to a hydroxy substituted carbon atom [7]. According to these spectroscopic data and the absence of a Cotton effect together with considerations of JA biosynthesis [7, 8] IRF-2 is probably identical with *N*-[(+)-cucurbinoyl]-*S*-tryptophan (**3**). This has to be confirmed by further investigations, e.g. partial synthesis.

The detection of these amino acid conjugates in broad bean flowers supports the assumption that conjugation is an important route in the metabolism of the growth regulators of the jasmonic acid type. Such amino acid conjugates are known also from other plant sources [9, 10].

EXPERIMENTAL

Plant material. The same as used in ref. [6].

Extraction and isolation. The extraction of *ca* 9 kg broad bean flowers and the subsequent purification procedure described in [6] gave, after preparative HPLC (system I), an immunoreactive fraction (IRF, 15.6 mg) less polar than *N*-[(−)-jasmonoyl]-*S*-tyrosine. Methylation of IRF by CH_2N_2 and HPLC purification (system II) yielded 9.9 mg IRF-Me. Subsequent HPLC (system III) of IRF-Me resulted in the separation of IRF-1-Me (3.5 mg, R_t = 50.3 min) and IRF-2-Me (1.7 mg, R_t = 55.4 min).

HPLC. According to ref. [6]. Solvent systems: (I). MeOH–H_2O (0.2% HOAc) = 55:45 (*v/v*), (II). MeOH–H_2O (0.2% HOAc) = 65:35 (*v/v*). For prep. HPLC (III) a reverse phase column Hypersil RP 8 (200 × 4.6 mm) was used. (III). MeOH–H_2O (0.2% HOAc) = 40:60 (*v/v*). Flow rate: 2 ml/min. UV detection: 228 nm.

Mass spectroscopy. The positive (10–16 eV) and negative (2–4 eV) ion mass spectra were obtained using an electron attachment mass spectrograph of the Research Institute 'Manfred von Ardenne', Dresden.

^{1}H *NMR.* 200.13 MHz, 400 MHz, $CDCl_3$, TMS as int. standard.

ORD. Jasco ORD/UV-5 spectropolarimeter.

N-[(−)-Jasmonoyl]-S-tryptophan methyl ester (**2**; *IRF-1-Me*). MS (10–16 eV) *m/z* (rel. int.): 410 $[M]^+$ (4), 378 (4), 201 $[d-H]^+$ (41), 170 (4), 159 (4), 143 (5), 130 $[b]^+$ (100), 117 $[a+H]^+$ (3). MS (negative ions; 2–4 eV) *m/z* (rel. int.): 409 $[M-1]^-$ (49), 377 (50), 350 (16), 341 $[e]^-$ (8), 279 $[c-H]^-$ (35), 248 (33), 234 (20), 201 $[d-H]^-$ (92), 200 (68), 142 (52), 129 $[b-H]^-$ (100), 116 $[a]^-$ (23). ^{1}H NMR (200.13 MHz): δ0.94 (3H, *t*, J = 7.5 Hz, H-12), 3.23–3.42 (2H, *m*, H-3'), 3.72 (3H, *s*, COOMe), 4.97 (1H, *m*, H-2'), 5.2–5.5 (2H, 2 *br m*, H-9, H-10), 5.93 (1H, *br d*, J = 8.1 Hz, NH), 6.95–7.6 (5H, indole), 8.13 (1H, *br s*, H-1"); ORD: $[\phi]_{315}$ −1457°, $[\phi]_{270}$ +2154° (MeOH; *c* 0.12).

IRF-2-methyl ester (**4**). MS (10–16 eV) *m/z* (rel. int.): 412 $[M]^+$ (6), 394 (2), 201 $[d-H]^+$ (74), 170 (6), 159 (7), 143 (8), 130 $[b]^+$ (100), 117 $[a+H]^+$ (3). MS (negative ions; 2–4 eV) *m/z* (rel. int.): 411 $[M-1]^-$ (34), 378 (8), 352 (22), 281 $[c-H]^-$ (26), 250 (15), 236 (47) 201 $[d-H]^-$ (100), 200 (59), 142 (59), 129 $[b-H]^-$ (50), 116 $[a]^-$ (17). ^{1}H NMR (400 MHz): δ0.96 (3H, *t*, J = 7.5 Hz, H-12), 3.26–3.37 (2H, *m*, H-3'), 3.71 (3H, *s*, COOMe), 3.85 (1H, *m*), 4.96 (1H, *m*, H-2'), 5.32–5.49 (2H, 2 *m*, H-9, H-10), 5.95 (1H, *br d*, J = 8.0 Hz, NH), 6.95 (1H, *s*, H-2"), 7.12 (1H, *ddd*, $J_{4",5"} = J_{5",6"}$ = 7.5 Hz, $J_{5",7"}$ = 1.0 Hz, *H-5"), 7.20 (1H, ddd, $J_{5",6"} = J_{6",7"}$ = 7.5 Hz, $J_{6",4"}$ = 1.0 Hz, *H-6"), 7.37 (1H, d, $J_{6",7"}$ = 7.5 Hz, $J_{5",7"}$ unresolved, *H-7"), 7.54 (1H, *d*, $J_{4",5"}$ = 7.5 Hz, $J_{4",6"}$ unresolved, *H-4"), 8.1 (1H, *br s*, H-1"). *Assigned in comparison to data reported in [11] for a derivative of IAA.

ORD. Positive plain curve (MeOH; *c* 0.09).

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