Phytochemistry, Vol. 25, No. 9, pp. 2236–2237, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

N-[(−)-JASMONOYL]-*S*-TYROSINE: A CONJUGATE OF JASMONIC ACID FROM *VICIA FABA*

CHRISTIAN BRÜCKNER, ROBERT KRAMELL, GERNOT SCHNEIDER, HANS-DIETER KNÖFEL, GÜNTHER SEMBDNER and KLAUS SCHREIBER

Institute of Plant Biochemistry, Academy of Sciences of the GDR, 4020 Halle, Weinberg 3, East Germany

(*Received* 8 *January* 1986)

Key Word Index—*Vicia faba*: Fabaceae: jasmonic acid: conjugate: *N*-[(−)-jasmonoyl]-*S*-tyrosine.

Abstract—*N*-[(−)-Jasmonoyl]-*S*-tyrosine has been isolated from flowers of *Vicia faba* and its structure elucidated by spectroscopic methods.

INTRODUCTION

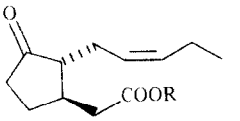
(−)-Jasmonic acid [(−)-JA, **1**] and its methyl ester [(−)-JA-Me, **2**] represent a new type of endogenous plant growth regulator widely distributed [1] and possessing interesting physiological properties [2]. In continuing investigations on the occurrence of (−)-JA in *Vicia faba* [3] we studied its distribution in different parts of the broad bean plant using an optimized RIA [4; Knöfel, H.-D. *et al.*, unpublished] in combination with TLC. Extracts of flowers contained, in addition to small amounts of (−)-JA, a polar substance exhibiting high immunoreactivity. Because of its high cross reactivity similar to synthetic JA amino acid conjugates [Kramell, R. *et al.*, unpublished results] and its polar behaviour, this compound was assumed to be a conjugate.

At present, only two native JA conjugates of the amino acid type are known [5, 6]. Here we describe the isolation and structural elucidation of a new amino acid conjugate of (−)-JA from broad bean flowers.

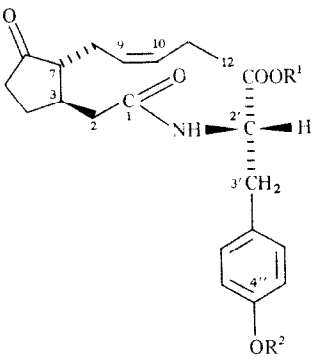
RESULTS AND DISCUSSION

Flowers of *Vicia faba* were extracted with methanol. After evaporation an acidic chloroform fraction was obtained and purified by DEAE-Sephadex A-25 chromatography. JA and a more polar immunoreactive substance (IRS) were detected by RIA in the eluted fractions. The following purification steps were monitored by RIA. Further purification was achieved by reverse phase column chromatography and repeated preparative HPLC (solvent systems I and II). Methylation of the purified IRS by diazomethane resulted in two immunoreactive fractions (IRS-Me-1 and IRS-Me-2) separated by HPLC (solvent system III) which were subsequently subjected to MS, ^{1}H NMR and IR.

In the mass spectrum of IRS-Me-1 ($[M]^+$ at *m/z* 387) key fragments at *m/z* 178 $[HOC_6H_4CHCHCO_2Me]^{\cdot+}$ and 107 $[CH_2C_6H_4OH]^{\cdot+}$ were observed which reveal the presence of a tyrosine moiety attached to the parent JA (**4**). Likewise, the mass spectrum of IRS-Me-2 ($[M]^+$ at *m/z* 401) showed corresponding fragments at *m/z* 192 $[MeOC_6H_4CHCHCO_2Me]^{\cdot+}$ and 121 $[CH_2C_6H_4OMe]^+$ coinciding with structure **5**. The proposed structures **4** and **5** were confirmed by data of ^{1}H NMR and IR spectroscopy. All spectra were found to be identical with those of synthetic *N*-[(−)-jasmonoyl]-*S*-tyrosine methyl ester (**4**) and its *O*-methyl ether (**5**) [Kramell, R. *et al.*, unpublished results]. From these results the structure of the isolated IRS was established as *N*-[(−)-jasmonoyl]-*S*-tyrosine (**3**).



1 R = H
2 R = Me



3 $R^1 = R^2 = H$
4 R^1 = Me, R^2 = H
5 $R^1 = R^2$ = Me

The detection of this new amino acid conjugate of the plant growth regulator JA in broad bean flowers is of high physiological interest, since conjugation of plant hormones is known to be a physiological relevant process in hormone metabolism [7].

EXPERIMENTAL

Plant material. Vicia faba L. cv. 'Fribo' was cultivated in a green house. Flowers (about 9 kg) were harvested in the stage of full flowering.

Extraction and isolation. Flowers were homogenized in 80% aq. MeOH. The filtered extracts were evaporated and the aq. phase was frozen, thawed and centrifuged at 6000 *g*. The supernatant was adjusted to pH 2.5 and extracted with $CHCl_3$ (10 × 2 l.). After evaporation the residue (about 4.7 g) was separated on a column (75 × 3 cm) of DEAE-Sephadex A-25 [8] using a gradient of HOAc in MeOH. Aliquots of each fraction were tested by RIA. JA was eluted with 0.5 N HOAc in MeOH and the IRS (about 100 mg) with 0.85 N HOAc in MeOH. The IRS fraction was subjected to CC (14 × 0.8 cm) with LiChroprep RP 18 using a discontinous gradient of MeOH in 0.2% aq. HOAc. The fraction eluted with 70–80% MeOH contained the IRS (about 66 mg). Final purification was achieved by repeated prep. HPLC (systems I and II) resulting in 7 mg IRS. Methylation of this fraction with CH_2N_2 and subsequent HPLC (system III) gave IRS-Me-1 (2.2 mg) and IRS-Me-2 (3.1 mg).

HPLC. For both analytical and prep. HPLC a reverse phase column (LiChrosorb RP 8, 250 × 4.6 mm) was used. Solvent systems: I, MeOH–H_2O (0.2% HOAc), 55:45 (v/v); II, MeOH–H_2O (0.2% HOAc), 45:55 (v/v); III, MeOH–H_2O (0.2% HOAc), 60:40 (v/v). Flow rate: 1 ml/min, UV detection: 240 nm.

Mass spectrometry. The mass spectra were obtained using an electron attachment mass spectrograph of the Research Institute 'Manfred von Ardenne', Dresden, East Germany, operating at 6–16 eV ionization energy.

^1H *NMR.* 200 MHz, $CDCl_3$, TMS as int. standard.

IR. Specord 75 IR (VEB Carl Zeiss, Jena, East Germany).

N-[(−)-*Jasmonoyl*]-S-*tyrosine methyl ester* (**4**; *IRS-Me*-1). MS *m/z* (rel. int.): 387 $[M]^+$ (21), 369 (3), 355 (26), 249 (19), 236 (12), 220 (5), 210 (17), 196 (13), 192 (11), 178 (95), 151 (19), 147 (24), 142 (12), 136 (22), 107 (100); ^1H NMR: δ 0.93 (3H, *t*, J = 7.5 Hz, H-12), 3.02 (2H, *m*, H-3'), 3.73 (3H, *s*, COOMe), 4.83 (1H, *m*, H-2'), 5.2–5.4 (2H, *br m*, H-9, H-10), 5.84 (1H, *br d*, J = 6.3 Hz, NH), 6.71 (2H, *d*, J = 8.5 Hz, arom. H), 6.93 (2H, *d*, J = 8.5 Hz, arom. H); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (phenol), 1730 (CO_2Me, C=O), 1670 (amide), 1510 (amide),1200 (arom.).

N-[(−)-*Jasmonoyl*]-*methoxy*-S-*tyrosine methyl ester* (**5**: *IRS-Me*-2). MS *m/z* (rel. int.): 401 $[M]^+$ (23), 383 (2), 369 (2), 251 (4), 220 (2), 210 (5), 192 (100), 161 (10), 151 (4), 150 (9), 142 (2), 134 (4), 121 (51); ^1H NMR: δ 0.94 (3H, *t*, J = 7.5 Hz, H-12), 3.05 (2H, *m*, H-3'), 3.73 (3H, *s*, COOMe), 3.76 (3H, *s*, OMe), 4.84 (1H, *m*, H-2'), 5.2–5.4 (2H, *br m*, H-9, H-10), 5.8 (1H, *br d*, J = 6 Hz, NH), 6.80 (2H, *d*, J = 8.7 Hz, arom. H), 6.98 (2H, *d*, J = 8.7 Hz, arom. H): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (CO_2Me, C=O), 1670 (amide), 1510 (amide), 1200 (arom.).

Acknowledgements—The authors are indebted to Dr. J. Schmidt for mass spectra and to Mrs. C. Gebhardt and Mrs. E.-M. Schneider for technical assistance.

REFERENCES

1. Meyer, A., Miersch, O., Büttner, C., Dathe, W. and Sembdner, G. (1984) *J. Plant Growth Regul.* **3**, 1.
2. Sembdner, G. and Klose, C. (1985) *Biol. Rdsch.* **23**, 29.
3. Dathe, W., Rönsch, H., Preiss, A., Schade, W., Sembdner, G. and Schreiber, K. (1981) *Planta* **153**, 530.
4. Knöfel, H.-D., Brückner, C., Kramell, R., Sembdner, G. and Schreiber, K. (1984) *Biochem. Physiol. Pflanz.* **179**, 317.
5. Cross, B. E. and Webster, G. R. B. (1970) *J. Chem. Soc.* (*C*) 1839.
6. Bohlmann, F., Wegner, P., Jakupovic, J. and King, R. M. (1984) *Tetrahedron* **40**, 2537.
7. Sembdner, G., Gross, D., Liebisch, H.-W. and Schneider, G. (1980) in *Hormonal Regulation of Development I* (MacMillan, J., ed.) Encyclopedia of Plant Physiology, Vol. 9. Springer, Berlin.
8. Gräbner, R., Schneider, G. and Sembdner, G. (1976) *J. Chromatogr.* **121**, 110.