Journal of Chromatography, 483 (1989) 459–462
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 955

Note

Separation of diastereomeric amino acid conjugates of jasmonic acid

GERNOT SCHNEIDER*, ROBERT KRAMELL and CHRISTIAN BRÜCKNER
Institute of Plant Biochemistry, Academy of Sciences of the G.D.R., Weinberg 3, 4050 Halle/Saale (G.D.R.)
(First received May 9th, 1989; revised manuscript received August 17th, 1989)

(−)-Jasmonic acid [(3*R*,7*R*,9*Z*)-jasmonic acid; (−)-JA] is an endogenous plant constituent that exhibits plant hormone-like activity[1,2]. In addition to (−)-JA and its methyl ester, JA amino acid conjugates have also been found in plants[3–5]. The structures of these conjugates were elucidated by gas chromatography–mass spectrometry (GC–MS) and NMR spectroscopy by comparison with synthetic standards[6]. These methods, however, are not suitable for establishing the stereochemistry of diastereomeric compounds.

This paper describes the separation of diastereomeric amino acid conjugates of JA by high-performance liquid chromatography (HPLC) in order to evaluate the stereochemical purity of synthetic specimens and to assign the appropriate stereochemistry to endogenous compounds.

EXPERIMENTAL

High-performance liquid chromatography

An HP 1090 liquid chromatograph was used in combination with a personal computer HP 85, an integrator HP 3395 and a data storage HP 9033. The photodiode-array detector was operated at 210 nm. A Hypersil RP-8 (5 μm) column (200 × 4.6 mm I.D.) was eluted isocratically with mixtures of methanol and water or aqueous 0.1% phosphoric acid[7].

Standard compounds

The standard compounds were synthesized from (±)-JA and the corresponding amino acids[6]. The structures of their chiral part are given in Fig. 1. Methanolic solutions of the compounds (1 mg/ml) were injected (5–20 μl).

Circular dichroism (CD)-measurement

The CD curve was measured with a JASCO J-20C automatic recording spectropolarimeter equipped with a DP-5 IN data processor. With 220 μg of (−)-JA–(*S*)-Ile in 1 ml of methanol with a 1-mm path length, an ellipticity of −0.002° at 296 nm was obtained.

The isolation and structural elucidation of (−)-JA–(*S*)-Trp from flowers and (−)-JA–(*S*)-Ile from apex leaves of *Vicia faba* were described elsewhere[5,8].

0021-9673/89/$03.50

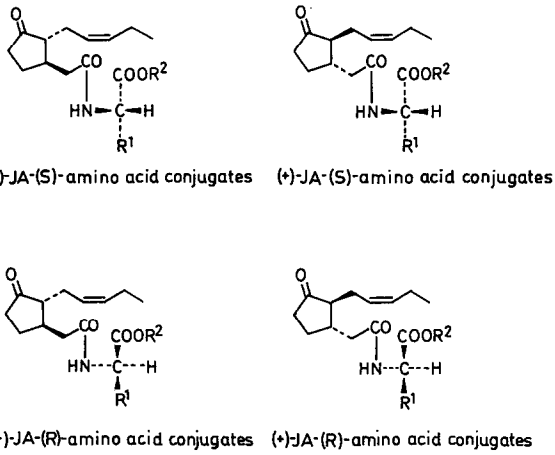

Fig. 1. Structures of the stereochemistry of diastereomeric N-(jasmonoyl)–amino acid conjugates.

TABLE I

RETENTION VOLUMES [V_R (ml)] AND RESOLUTION (R) OF DIASTEREOMERIC N-(JASMONOYL)–AMINO ACID CONJUGATES AND THEIR METHYL ESTERS ON HYPERSIL RP-8 WITH DETECTION AT 210 nm

Conjugate	*Free acids ($R^2 = H$), methanol–0.1% H_3PO_4 (40:60), 1 ml/min*		*Methyl esters ($R^2 = CH_3$), methanol–water (40:60), 1.5 ml/min*	
	V_R (ml)	*R*	*V_R (ml)*	*R*
(−)-JA–(*S*)-Val	12.2	4.00	16.7	2.63
(+)-JA–(*S*)-Val	9.2		13.5	
(−)-JA–(*R*)-Val	9.2	4.00	13.5	2.03
(+)-JA–(*R*)-Val	12.2		16.7	
(−)-JA–(*S*)-Ile	22.4	4.67	28.2	3.39
(+)-JA–(*S*)-Ile	16.0		22.4	
(−)-JA–(*S*)-Leu	25.2	5.06	31.2	3.85
(+)-JA–(*S*)-Leu	17.6		23.0	
(−)-JA–(*S*)-Phe	22.4	3.10	28.7	2.27
(+)-JA–(*S*)-Phe	17.8		24.0	
(−)-JA–(*R*)-Phe	17.7	3.20	23.9	2.38
(+)-JA–(*R*)-Phe	22.5		28.5	
(−)-JA–(*S*)-Trp	18.4	1.92	24.6	1.33
(+)-JA–(*S*)-Trp	16.3		22.1	
(−)-JA–(*S*)-Trp (isolated)	–		24.7	
(−)-JA–(*S*)-Asp	24.3[a]	1.44	37.2[b]	0.67
(+)-JA–(*S*)-Asp	22.4[a]		35.6[b]	
(−)-JA–(*S*)-Glu	29.4[a]	1.85	50.0[b]	0.79
(+)-JA–(*S*)-Glu	24.9[a]		45.6[b]	

[a] Methanol–0.1% H_3PO_4 (15:85), 1.5 ml/min.
[b] Methanol–water (25:75), 2 ml/min.

RESULTS AND DISCUSSION

HPLC data

The retention data obtained with eighteen JA–amino acid conjugates and their methyl esters on an RP-8 column are presented in Table I. As expected, the elution sequence (JA–Val, JA–Ile, JA–Leu) can be correlated with the chain length of the amino acid residue for the same diastereomer.

All diastereomeric pairs of a given enantiomer of the JA or an amino acid could clearly be separated, even with dicarboxylic amino acid conjugates such as JA–Asp or JA–Glu. As a rule, (+)-JA–(*S*)- and (−)-JA–(*R*)- conjugates elute before (+)-JA–(*R*)- and (−)-JA–(*S*)- conjugates. The best resolution was observed for diastereomeric JA–Leu conjugates (R = 5.06).

With the corresponding JA-amino acid methyl esters, the elution pattern of the acidic compounds remains, but the resolution declines.

The excellent separation of diastereomeric JA–amino acid conjugates achieved is useful for checking the stereochemical purity of synthetic compounds[6].

Stereochemical assignment of endogenous JA–amino acid conjugates

The separation of diastereomeric JA–amino acid conjugates by HPLC enables the complete stereochemistry of a compound to be assigned if the chirality of one component is known. Here, the stereochemistry of the JA moiety can easily be established by optical rotary dispersion or CD. Then, the HPLC retention can be used to assign the chirality of the adjacent amino acid, which is difficult to determine without hydrolysis.

From the apical leaves of *Vicia faba* a JA–amino acid conjugate has been isolated and elucidated by GC–MS and NMR to be JA–Ile[8]. By CD measurement, a negative Cotton effect at 296 nm [$a_{\text{endogenous}} = -39.6$ (the a value was calculated from θ according to $a = 0.0122\ \theta$) and $a_{\text{synthetic}} = -32.6$] was observed, from which only the JA counterpart could be assigned to be (−)-JA. From the HPLC retention it

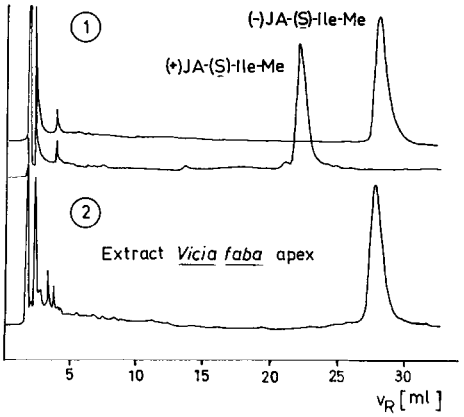

Fig. 2. HPLC traces of (1) synthetic diastereomers of JA–Ile methyl ester and (2) JA–Ile (methylated) isolated from *Vicia faba* on a Hypersil RP-8 (5 μm) column (200 mm × 4.6 mm I.D.) with methanol–0.1% H_3PO_4 (40:60) as eluent at 1.5 ml/min with detection at 210 nm.

could clearly be concluded (Fig. 2) that the attached amino acid has to be the (*S*)-enantiomer. Therefore, the stereochemistry of the isolated compound was determined to be (−)-JA–(*S*)-Ile.

Likewise, a (−)-jasmonoyl–tryptophan conjugate was isolated from flowers of *Vicia faba* (negative Cotton effect at 296 nm, $a_{\text{endogenous}} = -36.1$, $a_{\text{synthetic}} = -38.6$)[5]. HPLC of the methyl ester, the stereochemistry was determined to be (−)-JA–(*S*)-Trp (Table I).

ACKNOWLEDGEMENT

We are indebted to Dr. I. Yamaguchi, Department of Agricultural Chemistry, University of Tokyo, for measuring the CD curve of (−)-JA–(*S*)-Ile.

REFERENCES

1 H. Yamane, H. Takaji, H. Abe, T. Yokota and N. Takahashi, *Plant Cell Physiol.*, 22 (1981) 689.
2 A. Meyer, C. Miersch, C. Büttner, W. Dathe and G. Sembdner, *J. Plant Growth Regul.*, 3 (1984) 1.
3 F. Bohlmann, P. Wegner, J. Jakupovic and R. M. King, *Tetrahedron*, 40 (1984) 2537.
4 C. Brückner, R. Kramell, G. Schneider, H.-D. Knöfel, G. Sembdner and K. Schreiber, *Phytochemistry*, 25 (1986) 2236.
5 C. Brückner, R. Kramell, G. Schneider, J. Schmidt, A. Preiss, G. Sembdner and K. Schreiber, *Phytochemistry*, 27 (1988) 275.
6 R. Kramell, J. Schmidt, G. Schneider, G. Sembdner and K. Schreiber, *Tetrahedron*, 44 (1988) 5791.
7 G. Sembdner, G. Schneider and K. Schreiber (Editors) *Methoden zur Pflanzenhormonanalyse*, Springer, Berlin, Heidelberg, 1987, p. 112.
8 J. Schmidt, R. Kramell, C. Brückner, G. Schneider, J. Stach, E. Jensen, G. Sembdner and K. Schreiber, *Biomed. Environ. Mass Spectrom.*, submitted for publication.