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Short communication

Chromatographic resolution of peptide-like conjugates of jasmonic acid and of cucurbic acid isomers

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Abstract

The chiral separation of peptide-like conjugates of jasmonic acid and of cucurbic acid isomers was investigated by liquid chromatography on Chiralpak AS and Nucleodex β-PM. The retention sequences reflect distinct chromatographic properties with respect to the chirality of the jasmonic acid part or of the cucurbic acid isomers. The chromatographic behaviour of the amide conjugates on a reversed-phase C_{18} column provides evidence for the resolution of diastereomeric conjugates depending on the chirality of both constituents of the conjugate molecule. The chromatographic procedures are suitable for the analytical and preparative separation of such conjugates.

Keywords: Enantiomer separation; Jasmonic acid; Cucurbic acid

1. Introduction

(3*R*,7*R*)-(−)-Jasmonic acid [(−)-JA] and its methyl ester [(−)-JA–Me] are endogenously occurring plant growth regulators that are proposed to function as signal molecules in plant stress responses (for review, see [1,2]). The absolute configuration was determined on JA–Me and structural analogs by means of chiroptical and gas chromatographic analyses of endogenous and synthetic individuals [3,4]. Conjugates of (−)-JA with α-amino acids have been detected in several plant species and they also accumulate in plant tissues upon stress treatment [5,6]. Recently, a conjugate of JA with tyramine, a biogenic amine formed by decarboxylation of tyrosine, has been isolated from pollen of *Petunia* [7].

Structurally related compounds are (3*R*,6*S*,7*S*)-cucurbic acid (CA) and its 6,7-isomers containing a hydroxyl group instead of the keto function on the plane of the cyclopentane ring. They have also been detected as native compounds [5,8]. Previous investigations on *Pinus mugo* L. have shown the occurrence of the (*S*)-isoleucine conjugate of (3*R*,6*S*,7*R*)-cucurbic acid (7-iso-CA) [9].

Due to the fact that conjugates act as signals in a stereospecific manner [10,11], chirally defined compounds of JA and CA isomers are of interest. Furthermore, separation of isomers is necessary in the structural elucidation of native compounds.

Here, authentic substances were chemically synthesized, and the resulting isomeric conjugates were subjected to high-performance liquid chromatography using reversed-phase conditions (RP-HPLC), as well as the chiral phases (CP-HPLC) Chiralpak AS and Nucleodex β-PM to elucidate complete separation procedures.

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2. Experimental

2.1. Chromatographic conditions

HPLC was carried out on a Hewlett-Packard 1090 liquid chromatograph at a flow-rate of 1 ml/min with UV detection at 210 nm. For the analyses, the following HPLC columns (250×4.6 mm I.D., 5 μm particle size) were used: LiChrospher 100 RP-18 (Merck, Darmstadt, Germany), Chiralpak AS (Daicel J.T. Baker, Gross Gerau, Germany), Nucleodex β-PM (Macherey-Nagel, Düren, Germany).

2.2. Authentic substances

The standard compounds were prepared by reaction of racemic (3*R*,7*R*/3*S*,7*S*)-(±)-JA with (*S*)- or (*R*)-isoleucine, (*S*)-leucinol and the biogenic amine tyramine according to procedures described elsewhere [12,13]. The cucurbic acid conjugates were obtained from the respective conjugate of jasmonic acid by treatment with sodium boronate [14]. Starting from *N*-[(−)-jasmonoyl]–(*S*)-isoleucine, the reduction provided epimeric compounds of the 6,7-CA isomers called 7-iso-CA and 6-*epi*-7-iso-CA. The reduction of *N*-[(+)-jasmonoyl]–(*S*)-isoleucine resulted in the epimeric conjugates of the 3,6-CA isomers 3-iso-CA and 3-iso-6-*epi*-CA (Fig. 1).

3. Results and discussion

3.1. RP-HPLC

The retention of the amino acid conjugates of

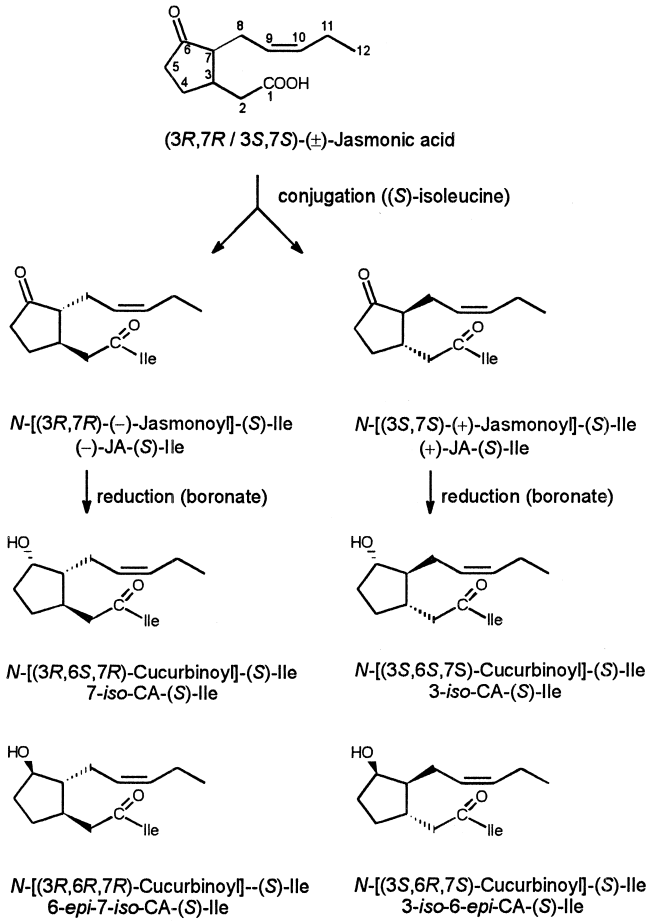

Fig. 1. Structures of jasmonic acid, *N*-(jasmonoyl)–(*S*)-isoleucine conjugates and *N*-(cucurbinoyl)–(*S*)-isoleucine conjugates.

jasmonic acid was found to depend on the chirality of both constituents of the conjugate molecule. Interestingly, diastereomeric pairs containing a polar carboxyl or hydroxyl group in the conjugate molecule could be baseline separated under reversed-phase conditions (Table 1). The diastereomeric pair (3*R*,7*R*/3S,7*S*)-(±)-JA–(*S*)-isoleucine was resolved with the longer retention time of the natural form (3*R*,7*R*)-(−)-JA–(*S*)-Ile (Fig. 2A). The corresponding diastereomeric pair with the opposite chirality, (*R*)-isoleucine, was also separated into the diastereomers. However, in this case, the elution pattern was reversed (Fig. 2C). As expected, no resolution could be obtained for the enantiomeric compounds (3*R*,7*R*)-JA–(*S*)-Ile and (3*S*,7*S*)-JA–(*R*)-Ile as well as (3*R*,7*R*)-JA–(*R*)-Ile and (3*S*,7S)-JA–(*S*)-Ile (Table 1).

Table 1
Retention times ([t_R (min)] and resolutions (R_S) of peptide-like conjugates of jasmonic acid and of cucurbic acid isomers, respectively, obtained by RP-HPLC and CP-HPLC*

Compound	RP-18		Chiralpak AS	
	t_R (min)	R_S	t_R (min)	R_S
(+)-JA–(*S*)-Ile	15.6[a]		7.3[c]	
		3.49		3.62
(−)-JA–(*S*)-Ile	18.5[a]		5.3[c]	
(+)-JA–(*R*)-Ile	18.4[a]		6.3[c]	
		3.32		1.56
(−)-JA–(*R*)-Ile	15.6[a]		5.5[c]	
(+)-JA–(*S*)-leucinol	10.7[a]		19.4[d]	
		5.25		1.15
(−)-JA–(*S*)-leucinol	13.6[a]		16.3[d]	
(+)-JA–tyramine	16.5[b]		49.2[e]	
		0		1.80
(−)-JA–tyramine	16.5[b]		39.0[e]	
3-iso-CA–(*S*)-Ile	33.0[b]		80.4[f]	
		1.24		1.04
3-iso-6-*epi*-CA–(*S*)-Ile	30.6[b]		72.7[f]	
7-iso-CA–(*S*)-Ile	37.8[b]		57.3[f]	
		1.16		1.72
6-*epi*-7-iso-CA–(*S*)-Ile	40.4[b]		66.3[f]	

* Mobile phases:
[a] Methanol–0.2% (v/v) aqueous acetic acid (60:40, v/v).
[b] Methanol–0.2% (v/v) aqueous acetic acid (50:50, v/v).
[c] *n*-Hexane–2-propanol (70:30, v/v).
[d] *n*-Hexane–2-propanol (95:5, v/v).
[e] *n*-Hexane–2-propanol (80:20, v/v).
[f] *n*-Hexane–2-propanol (98:2, v/v).

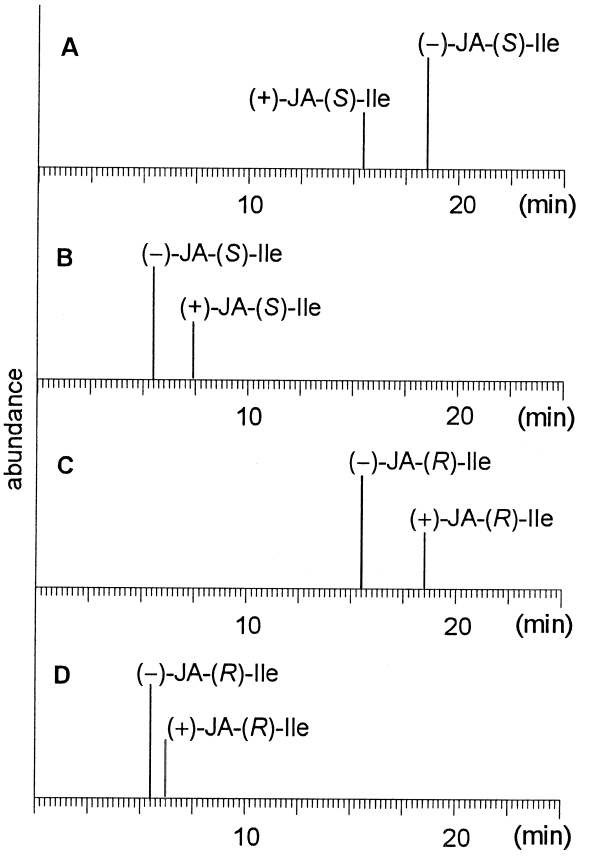

Fig. 2. Schematic presentation of the retention of JA conjugated with (*S*)- and (*R*)-isoleucine by RP-HPLC (A, C) and CP-HPLC on Chiralpak AS (B, D). For conditions, see Section 2 and Table 1.

The chromatographic properties of JA conjugates containing (*S*)-leucinol were identical to those found for the conjugates of the (*S*)-amino acids with the JA enantiomers with respect to resolution as well as elution sequence (Fig. 3A).

The enantiomeric pair of (3*R*,7*R*/3*S*,7*S*)-(±)-JA–tyramine was not separable on the non-chiral matrix (Fig. 4A).

Among cucurbic acid derivatives, the isomeric *N*-(cucurbinoyl)–(*S*)-isoleucines exhibited an elution order similar to those of the respective JA compounds. The diastereomeric conjugates of the 6,7-CA isomers derived from (3*R*,7*R*)-JA eluted after the corresponding isoleucine conjugates of the 3,6-CA isomers originating from (3*S*,7*S*)-JA (Fig. 5A). Interestingly, among the epimeric conjugates of the natural 6,7-CA isomers, 7-iso-CA–(*S*)-Ile eluted

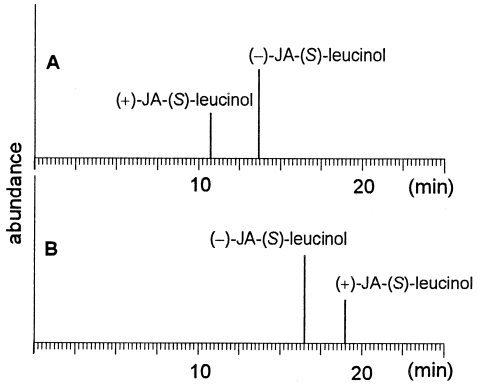

Fig. 3. Schematic presentation of retentions of JA conjugated with (*S*)-leucinol by RP-HPLC (A) and CP-HPLC on Chiralpak AS (B). For conditions, see Section 2 and Table 1.

earlier than its 6-epimer. This is in contrast with the inverse elution sequence detected by the RP-HPLC of the 6,7-CA isomers applied as free acids or methyl esters [8].

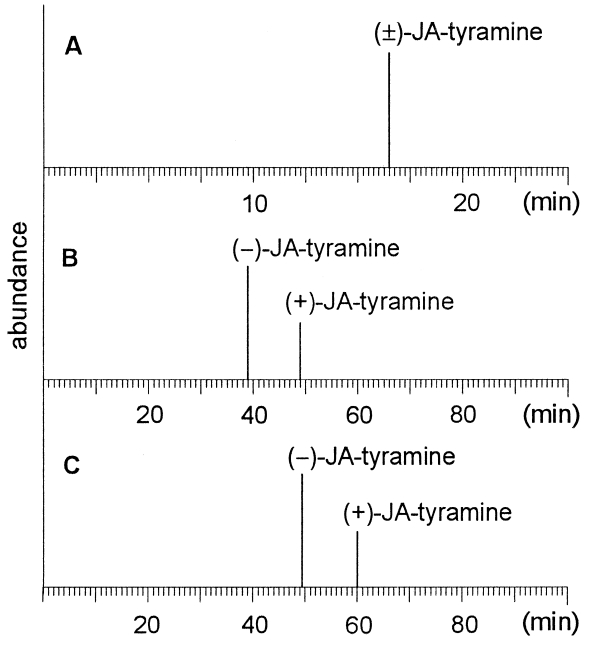

Fig. 4. Schematic presentation of the retention of enantiomeric JA conjugated with tyramine by RP-HPLC (A) and by CP-HPLC on Chiralpak AS (B) or Nucleodex β-PM (C). For conditions, see Section 2 and Table 1. (C) mobile phase, methanol–0.1% (v/v) triethylammonium acetate buffer, pH 4.7 (25:75, v/v); retention times [t_R (min)]: (−)-JA–tyramine=49.4, (+)-JA–tyramine= 60.1; resolution (R_S): 1.13.

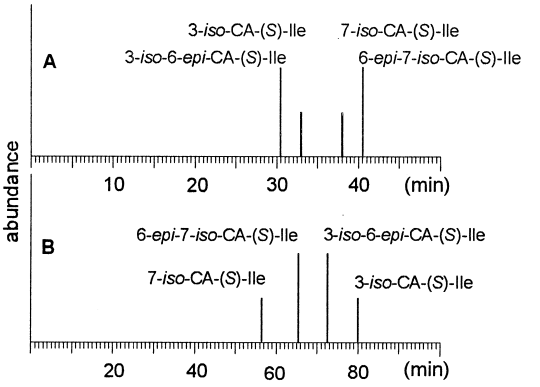

Fig. 5. Schematic representation of the retention of cucurbic acid isomers conjugated with (*S*)-isoleucine by RP-HPLC (A) and CP-HPLC on Chiralpak AS (B). For conditions, see Section 2 and Table 1.

3.2. CP-HPLC

The chiral stationary phases Chiralpak AS or Nucleodex β-PM were successfully used to resolve the enantiomers of JA, JA–Me or 12-hydroxy-JA–Me [15,16]. Here, we show that, for the peptide-like JA conjugates, there is a strong dependency of elution pattern on the chirality of the JA part. Within this dependency, conjugates of (3*R*,7*R*)-JA eluted earlier than the corresponding (3*S*,7*S*)-JA derivatives, and the diastereomeric pairs obtained by conjugation of racemic JA with (*S*)-isoleucine (Fig. 2B), (*R*)-isoleucine (Fig. 2D) and (*S*)-leucinol (Fig. 3B) could be baseline separated into their diastereomers (Table 1). In contrast to common RP-HPLC, the chiral conditions also allow complete separation of the enantiomeric conjugates, such as (3*R*,7*R*)-JA–(*S*)-Ile and (3*S*,7*S*)-JA–(*R*)-Ile (Table 1). Both differ significantly in their retention properties.

Furthermore, the enantiomeric pair of racemic JA and tyramine was completely resolved on the chiral stationary phase Chiralpak AS (R_S, 1.8) (Fig. 4B). On the cyclodextrin column, Nucleodex β-PM, the selectivity towards the optical antipodes was lower, as indicated by the resolution factor (R_S, 1.13) (Table 1). With respect to the elution sequence, on Chiralpak AS, the epimeric 6,7-CA isomers appeared before the epimeric 3,6-CA isomers (Fig. 5B). This accords with data found for the JA–(*S*)-isoleucine derivatives (Fig. 2B). In contrast, among the enantio-

meric forms of JA–Me, the (3*S*,7*S*)-(+)-JA–Me was eluted before its chiral counterpart using Chiralpak AS [15]. Interestingly, on the cyclodextrin phase, Nucleodex β-PM, identical elution sequences were observed for jasmonates applied as amides, methyl esters [16] or free acids [16]. Although the chromatographic behaviour shown here suggests a distinct role of the stationary phase, further investigations are necessary to explain the selectivity of the used chiral phases in resolving isomeric jasmonates.

The chromatographic data indicate that RP-HPLC is a suitable tool for separating chiral substances of peptide-like conjugates that contain JA or CA isomers and enantiomeric amino acids or amino alcohols. The chiral separations demonstrate that Chiralpak AS can be used for the stereochemical identification of peptide-like conjugates of jasmonic acid and cucurbic acid isomers originating from synthetic or natural sources. The chromatographic modes allow the separation of such compounds of JA and CA isomers on both the analytical and the preparative scale.

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References

[1] C. Wasternack, B. Parthier, Trends Plant Sci. 2 (1997) 302.
[2] R.A. Creelmann, J.E. Mullet, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48 (1997) 355.
[3] R.K. Hill, A.G. Edwards, Tetrahedron 21 (1965) 1501.
[4] T. Kitahara, M. Inoue, S. Tamogami, R. Kaiser, Tetrahedron 52 (1996) 1487.
[5] G. Sembdner, B. Parthier, Annu. Rev. Plant Physiol. Plant Mol. Biol. 44 (1993) 569.
[6] R. Kramell, R. Atzorn, G. Schneider, O. Miersch, C. Brückner, J. Schmidt, G. Sembdner, B. Parthier, J. Plant Growth Regul. 14 (1995) 29.
[7] O. Miersch, H.D. Knöfel, J. Schmidt, R. Kramell, B. Parthier, Phytochemistry 47 (1998) 327.
[8] W. Dathe, C. Schindler, G. Schneider, J. Schmidt, A. Porzel, E. Jensen, I. Yamaguchi, Phytochemistry 30 (1991) 1909.
[9] H.D. Knöfel, G. Sembdner, Phytochemistry 38 (1995) 569.
[10] S. Tamogami, R. Rakwal, O. Kodama, FEBS Lett. 401 (1997) 239.
[11] T. Krumm, K. Bandemer, W. Boland, FEBS Lett. 377 (1995) 523.
[12] R. Kramell, J. Schmidt, G. Schneider, G. Sembdner, K. Schreiber, Tetrahedron 44 (1988) 5791.
[13] R. Kramell, G. Schneider, O. Miersch, Chromatographia 45 (1997) 104.
[14] R. Kramell, A. Porzel, O. Miersch, G. Schneider, Phytochem. Anal. 10 (1999) 821.
[15] M. Okamoto, H. Nakazawa, Biosci. Biotech. Biochem. 56 (1992) 1172.
[16] R. Kramell, G. Schneider, O. Miersch, Phytochem. Anal. 7 (1996) 209.