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Review

Separation of a new type of plant growth regulator, jasmonates, by chromatographic procedures

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ABSTRACT

Jasmonic acid and its related compounds are short-chain alkylcyclopentanone or alkylcyclopentane carboxylic acids and their derivatives, and have been recognized as a new type of plant growth regulator because of their wide occurrence in the plant kingdom together with abscisic acid-like physiological activities at low concentrations. These compounds each have two enantiomeric and diastereomeric forms due to the presence of the two chiral centres in the cyclopentanone or cyclopentane ring. For this reason, the separation of jasmonates is relatively difficult. This review surveys the experimental conditions for the separation of jasmonates using column chromatography, thin-layer chromatography, gas chromatography and high-performance liquid chromatography and some other techniques in the purification procedure based on their chemical properties. Qualitative and quantitative analyses of jasmonates using combined gas chromatography–mass spectrometry and with selected-ion monitoring are also described.

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1. INTRODUCTION. DISCOVERY OF JASMONATES AND THEIR OCCURRENCE IN THE PLANT KINGDOM

Jasmonic acid and its related compounds are widely distributed in the plant kingdom [1,2] and have been recognized as a new type of plant hormone-like growth regulator. The methyl ester form of jasmonic acid, methyl jasmonate, was first isolated from the essential oil of jasmine (*Jasminum gradiflorum* L.) in 1962 [3] and from Tunisian rosemary (*Rosmarinus officinalis* L.) in 1967 [4] as an odoriferous compound. The structure of this compound was elucidated but no biological activity was found at that time. In 1971, jasmonic acid was first isolated from the culture filtrates of *Lasiodiplodia theobromae* (the synonym of *Botryodiplodia theobromae* Pat.) as a plant growth inhibitor [5]. This was the first report that jasmonate has plant growth regulating activity. In 1980, methyl jasmonate was isolated and identified as a senescence-promoting factor of plant tissues [6], indicating that jasmonates have distinctive biological activities in plant growth and development. Since then there have been numerous reports on the physiological effects of jasmonates, including the inhibition of seed [7] and pollen [8] germination, stem growth [9], cell expansion and multiplication [10] and the promotion of senescence [6,11,12], abscission [13,14] and tuber formation [15,16]. Further, it has been found that jasmonates affect plant gene expression and regulation [17–19]. Judging from this evidence together with their wide distribution in the plant kingdom, jasmonates may act as significant plant hormones in almost all aspects of physiological phenomena in the life cycle of plants in a similar manner to abscisic acid (ABA).

In this paper, we review chromatographic procedures for the separation of jasmonic acid and related compounds, as used for purification during their isolation.

2. CHEMICAL PROPERTIES OF JASMONATES

The structures of naturally occurring jasmonic acid and related compounds are shown in Fig. 1. Jasmonates belong to cyclopentane compounds with a keto or hydroxyl group at the C-6 position (C-3 in the numbering of the cyclopentane ring), a carboxylic acid moiety at the C-3 (C-1) position and 2′-*cis*-pentenyl or other alkyl substituents at the C-7 (C-2) position. Jasmonic acid is chemically similar to ABA in molecular mass, solubility properties and p*K*.

As shown in Fig. 2, conjugate forms of jasmonates have also been isolated and their structures have been elucidated. They conjugate with

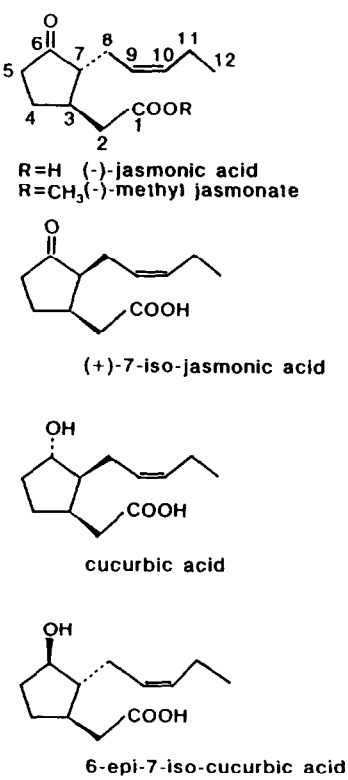
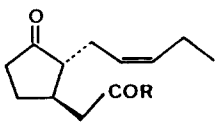
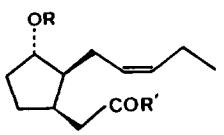


Fig. 1. Structural formulae of jasmonic acid and related compounds.



R= tyrosine N-[(-)-jasmonoyl]-S-tyrosine

R= tryptophan N-[(-)-jasmonoyl]-S-tryptophan



R= ß-D-glucose
R'= OH O-ß-D-glucopyranosylcucurbic acid

R= H
R'= tryptophan N-[(+)-cucurbinoyl]-S-tryptophan

Fig. 2. Structural formulae of conjugated jasmonates.

amino acid or glucose moieties. 3-O-β-D-Glucopyranosylcucurbic acid and its methyl ester, which have a reduced type of keto function at the C-6 (C-3) position, have been found in seeds of *Cucurbita pepo* L. [20–22]. 3-Oxo-2-(5′-β-D-glucopyranosyloxy- 2′ -pentenyl)cyclopentane-1-acetic acid (tuberonic acid O-β-D-glucoside) has been isolated from potato leaves as a specific tuber-inducing stimulus [16,23]. It has also been found that (±)-9,10-dihydrojasmonic acid is mainly converted into the O(11)-β-D-glucopyranoside of the 11-hydroxy derivative in a feeding experiment using barley seedlings [24]. Jasmonic acid conjugated with an amino acid moiety has been found in plant tissues [25–27] and fungi [28], whereas its glucosyl ether and glucosyl ester have not, although their existence is to be expected. The β-D-glucopyranosyl ester of jasmonic acid has been synthesized and was very unstable under acidic and alkaline conditions [29].

Jasmonic acid is biosynthesized from linolenic acid via the formation of 12-oxo-10(*Z*),15(*Z*)-phytodienoic acid in *Vicia faba* L. pericarp and other plant tissues [30,31]. Therefore, jasmonic acid and its related compounds each have two enantiomeric and diastereomeric forms due to the presence of the two chiral centres in the cyclopentanone or cyclopentane ring. The naturally occurring *cis* forms (3*R*,7*R*-forms) of jasmonic acid and its related compounds are known to be rapidly transformed into the *trans* forms by 7-epimerization (2-epimerization) during isolation procedures and other treatments, resulting in an equilibrium molar ratio of *trans* to *cis* forms of 9:1.

3. DETECTION OF JASMONATES

Detection of jasmonates has been based on their inhibitory effects in bioassays or their chemical properties.

A seed germination test on lettuce seeds [7], growth inhibition test on rice or lettuce seedlings [7] or chlorophyll degradation test on oat leaf segments [6] is recommended as a bioassay for the detection of jasmonates in extracts of plant tissues.

One can also detect jasmonates on thin-layer plates by spraying with anisaldehyde reagent, which consists of acetic acid–sulphuric acid–anisaldehyde (100:2:1) [32], with a 5% solution of vanillin in concentrated sulphuric acid [20] or with a 1% potassium permanganate solution [20] or by exposure to iodine vapour [33] (see Table 5). These compounds are also detected under UV radiation as fluorescent spots after spraying with 5% H_2SO_4–EtOH followed by heating at 130°C for 5 min [20].

4. EXTRACTION AND SOLVENT FRACTIONATION OF JASMONATES

In general, the concentrations of jasmonic acid and related compounds in plant tissues are low, as are those of plant hormones. Therefore, if one wants to isolate or identify these substances in a certain plant, the separation of the plant tissues from other parts is necessary prior to solvent extraction.

Plant materials containing jasmonates should be homogenized in a blender using a water-miscible organic solvent such as ethanol, methanol or acetone several times and then solvent extraction should be carried out in the usual way [34]. After the evaporation of the organic solvent at low temperature *in vacuo*, the aqueous solution should be partitioned with an organic solvent based on the distribution coefficient between the organic and aqueous phases. Free

forms of jasmonates are easily extracted with organic solvents because they are non-polar. As the acidic form of jasmonates is a short-chain alkylcyclopentanone or alkylcyclopentane carboxylic acid of $pK_a = 4–5$, the aqueous solution is adjusted to pH 2–3 with HCl and partitioned with ethyl acetate [7,35], diethyl ether [32,36], benzene [8] or chloroform [37,38]. If one wants to isolate jasmonates conjugated with amino acid moieties, chloroform can be recommended as the organic solvent for solvent fractionation [25,26].

5. CHROMATOGRAPHIC SEPARATION OF JASMONATES

5.1. Column chromatography

Column chromatography is a powerful purification technique for the isolation and the identification of jasmonates. In the purification of the acidic form of jasmonates, adsorption column chromatography on charcoal [7,33] or silica gel [5,36,39,40], partition column chromatography on silicic acid impregnated with formic acid [7,35,41] and ion-exchange column chromatography on DEAE-Sephadex A-25 [32,40] have been used. On the other hand, the neutral form can easily be purified by adsorption column chromatography on charcoal [6] or silicic acid [6]. DEAE-Sephadex A-25 column chromatography has frequently been applied to the purification of conjugate jasmonates [25,26], as also has adsorption column chromatography on silicic acid and Celite [20–22,28]. Supports and solvent systems used in column chromatography for the purification of jasmonates are given in Tables 1–3.

5.2. Thin-layer chromatography (TLC)

TLC has frequently been used for the purification and identification of jasmonates. In Table 4,

TABLE 1

ADSORPTION COLUMN CHROMATOGRAPHY OF JASMONATES

Compound	Adsorbent	Solvent system	Ref.
Jasmonic acid	Charcoal	35–65% aqueous acetone	7
	Charcoal	80% aqueous acetone	35
	Silica gel	Benzene–$CHCl_3$ (1:1)	5
	Silica gel L	$CHCl_3$–EtOAc (9:1)	36
	Silica gel	$CHCl_3$–MeOH (95:5)	39
	Silica gel	EtOAc–$CHCl_3$ (3:7–7:3)	40
Methyl jasmonate	Charcoal	70–80% aqueous acetone	6
	Silica gel (Wako-gel C 100)	Benzene–EtOAc (95:5)	6
	Silica gel (Wako-gel C 100)	*n*-Hexane–EtOAc (90:10)	6
Cucurbic acid	Silicic acid–Celite	Benzene–EtOAc (1:1)	20–22
	Charcoal–Celite	40–45% aqueous acetone	20–22
6-Epicucurbic acid	Silica gel	EtOAc–$CHCl_3$ (3:7–7:3)	40
7-Isocucurbic acid	Silica gel	EtOAc–$CHCl_3$ (3:7–7:3)	40
6-Epi-7-isocucurbic acid	Silica gel	EtOAc–$CHCl_3$ (3:7–7:3)	40
N-Jasmonoylisoleucine	Silica gel–Celite (1:2)	EtOAc–light petroleum (3:2)	27
N-Dihydrojasmonoylisoleucine	Silica gel–Celite (1:2)	EtOAc–light petroleum (3:2)	27
Cucurbic acid glucoside	Silicic acid–Celite	EtOAc–MeOH (90:10)	20–22
Cubcurbic acid glucoside methyl ester	Silicic acid–Celite	EtOAc–MeOH (90:10)	20–22

TABLE 2

PARTITION COLUMN CHROMATOGRAPHY OF JASMONATES

Compound	Support	Stationary phase	Mobile phase	Ref.
Jasmonic acid	Silicic acid	0.5 *M* formic acid	*n*-Hexane–EtOAc (99:1)	7, 35, 41
Cucurbic acid	Celite	1 *M* phosphate buffer (pH 5.4)	Benzene–*n*-butanol (97.5:2.5–95:5)	20–22
	Sephadex LH-20–Celite	1 *M* phosphate buffer (pH 5.4)	Benzene–*n*-butanol (97:3)	20–22
Cucurbic acid glucoside	Celite	1 *M* phosphate buffer (pH 5.4)	Benzene–*n*-butanol (80:20–70:30)	20–22
Cucurbic acid glucoside methyl ester	Celite	1 *M* phosphate buffer (pH 5.4)	Benzene–*n*-butanol (90:10–85:15)	20–22

TABLE 3

ION-EXCHANGE, GEL PERMEATION AND REVERSED-PHASE COLUMN CHROMATOGRAPHY OF JASMONATES

Compound	Support	Solvent system	Ref.
Jasmonic acid	DEAE-Sephadex A-25	80% aqueous MeOH–AcOH (98.5:1.5)	32, 36, 40
	Sephadex LH-20	MeOH	39
	Silica gel ODS-SQ_3	MeOH	33
	Silanized silica gel RP-2	$CHCl_3$–EtOAc (9:1)	37
(+)-7-Isojasmonic acid	Silanized silica gel RP-2	$CHCl_3$–EtOAc (9:1)	37, 42
(−)-9,10-Dihydrojasmonic acid	Silanized silica gel RP-2	$CHCl_3$–EtOAc (9:1)	37
3,7-Didehydrojasmonic acid	Silanized silica gel RP-2	$CHCl_3$–EtOAc (4:1)	37
4,5-Didehydrojasmonic acid	DEAE-Sephadex A-25	AcOH in 80% aqueous MeOH[a]	32
(+)-6-Epi-7-isocucurbic acid	DEAE-Sephadex A-25	AcOH in 80% aqueous MeOH[a]	32
	Silanized silica gel RP-2	$CHCl_3$–EtOAc (4:1)	37
N-[(−)-Jasmonoyl]-S-tyrosine	DEAE-Sephadex A-25	0.85 *M* AcOH in MeOH	25, 26
N-[(−)-Jasmonoyl]-S-tryptophan	DEAE-Sephadex A-25	0.85 *M* AcOH in MeOH	25, 26
N-[(+)-Cucurbinoyl]-S-tryptophan	DEAE-Sephadex A-25	0.85 *M* AcOH in MeOH	25, 26
(−)-9,10-Dihydro-11-hydroxyjasmonic acid O-β-D-glucopyranoside	DEAE-Sephadex A-25	0.5 *M* AcOH in MeOH	24
(+)-Jasmonic acid-β-D-glucosyl ester	Silanized silica gel	$CHCl_3$–EtOAc with increasing EtOH	29
Tuberonic acid O-β-D-glucopyranoside	Dowex 1 × 4 (CH_3COO^- form)	2 M AcOH	23
	Sephadex LH-20	10% or 30% MeOH	23
	LiChroprep RP-8	50% MeOH	23

[a] Discontinuous gradient.

adsorbents, developing solvent systems and R_F values are presented. Silica gel is the most commonly used support; in some instances other materials such as alumina have been used [36].

Jasmonates can be detected on thin-layer plates by spraying with reagents (Table 5). However, jasmonates cannot be detected as a quenching spot against a green fluorescent background because they do not show significant UV absorption.

TABLE 4

THIN-LAYER CHROMATOGRAPHY OF JASMONATES

Compound	Support	Solvent system	R_F [a]	Ref.
Jasmonic acid	Kieselgel GF_{254}	EtOAc–*n*-hexane–$CHCl_3$–AcOH (20:15:8:1)	–	7
	Kieselgel $60F_{254}$, 0.5 mm	*n*-hexane–EtOAc–AcOH (24:6:1)	–	8
	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.56	32
	Silica gel $60F_{254}$	Toluene–EtOAc–AcOH (80:10:4)	0.34	33
	Silica gel $60F_{254}$	2-Propyl alcohol–ammonia–water (10:1:1)	0.56	33
	Silica gel $60F_{254}$	Benzene–EtOAc–AcOH (10:1:1)	0.43–0.50	35
	Silica gel $60F_{254}$	*n*-Hexane–EtOAc–AcOH (10:1:1)	0.34–0.48	35
	Silica gel GF_{254}	Benzene–EtOAc–acetone–AcOH (40:10:5:1)	0.50–0.70	36
	Silica gel	Benzene–EtOAc (1:1)	0.35	39
	Silica gel	$CHCl_3$–MeOH (9:1)	0.39	39
	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.70	40
	Silica gel $60F_{254}$, 0.25 mm	*n*-Hexane–EtOAc–AcOH (10:1:1)	0.28–0.36	41
7-Isojasmonic acid	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.70	40
	Silica gel	$CHCl_3$–MeOH–AcOH (70:10:0.5)	0.22	43
4,5-Didehydro-jasmonic acid	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.56	32
4,5-Didehydro-7-isojasmonic acid	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5.1)	0.56	32
	Silica gel GF_{254}	*n*-Hexane–EtOAc–AcOH (60:40:1)	0.42	38
	Silica gel GF_{254}	$CHCl_3$–MeOH–AcOH (140:20:1)	0.20	38
(+)-11,12-Didehydro-7-isojasmonic acid	Silica gel	$CHCl_3$–MeOH–AcOH (70:10:0.5)	0.20	43
(−)-9,10-Dihydro-jasmonic acid	Silica gel, 1 mm	*n*-Hexane–EtOAc–AcOH (60:40:1)	0.45	37
(+)-9,10-Dihydro-7-isojasmonic acid	Silica gel	$CHCl_3$–MeOH–AcOH (70:10:0.5)	0.23	43
3,7-Didehydro-jasmonic acid	Silica gel, 1 mm	*n*-Hexane–EtOAc–AcOH (60:40:1)	0.47	37
Cucurbic acid	Silica gel G, 0.25 mm	Isopropyl ether–AcOH (95:5)	0.52	20–22
	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.36	32
	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.45	40
6-Epicucurbic acid	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.64	40
7-Isocucurbic acid	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.59	40
6-Epi-7-iso-cucurbic acid	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.42	32
	Silica gel, 1 mm	*n*-Hexane–EtOAc–AcOH (60:40:1)	0.32	37
	Silica gel, 1 mm	$CHCl_3$–MeOH–water (140:20:1)	0.17	37
	Silica gel GF_{254}, 0.3 mm	$CHCl_3$–EtOAc–acetone–AcOH (40:10:5:1)	0.52	40
	Silica gel	$CHCl_3$–MeOH–AcOH (70:10:0.5)	0.13	43
(+)-3-Oxo-2-(pentenyl)cyclopent-1-yl-propionic acid	Silica gel GF_{254}	$CHCl_3$–MeOH–AcOH (140:20:1)	0.25	38
	Silica gel GF_{254}	*n*-Hexane-EtOAc–AcOH (60:40:1)	0.50	38
(+)-3-Oxo-2-(pentenyl)cyclopent-1-yl-butyric acid	Silica gel GF_{254}	$CHCl_3$–MeOH–AcOH (140:20:1)	0.27	38
	Silica gel GF_{254}	*n*-Hexane–EtOAc–AcOH (60:40:1)	0.68	38
Methyl jasmonate	Kieselgel $60F_{254}$, 0.5 mm	Benzene–EtOAc (9:1)	–	8
	Silica gel PF_{254}, 0.5 mm	*n*-Hexane–EtOAc (5:1)	0.27–0.37	6
	Silica gel PF_{254}, 0.5 mm	Benzene–EtOAc (10:1, multiple)	0.30–0.34	6
	Al_2O_3	Benzene–EtOAc (3:2)	0.72	36
	Silica gel G	Benzene–EtOAc (3:2)	0.59	36
	Silica gel G	Diethyl ether	0.65	36
	Silica gel $60F_{254}$, 0.25 mm	*n*-Hexane–EtOAc (5:1)	0.59–0.72	41
Ethyl (+)-7-iso-jasmonate	Silica gel GF_{254}	$CHCl_3$–MeOH–AcOH (140:20:1)	0.91	38
	Silica gel GF_{254}	*n*-Hexane–EtOAc–AcOH (60:40:1)	0.68	38
Cucurbic acid glucoside	Silica gel G, 0.25 mm	$CHCl_3$–MeOH (85:15)	0.51	20–22
Cucurbic acid glucoside methyl ester	Silica gel G, 0.25 mm	$CHCl_3$–MeOH–AcOH (75:20:5)	0.60	20–22

[a] Dashes indicate data not given.

TABLE 5
COLOUR REACTIONS OF JASMONATES ON THIN-LAYER CHROMATOGRAPHY

Compound	Support	Reagent	Colour of spot	Ref.
Jasmonic acid	Silica gel GF_{254}, 0.3 mm	Anisaldehyde: heat[a]	Reddish brown	32
Cucurbic acid	Silica gel G, 0.25 mm	EtOH–H_2SO_4: heat[b] (UV)	Orange (pale yellow)	20
	Silica gel G, 0.25 mm	Vanillin–H_2SO_4: heat[c]	Blue green	20
6-Epi-7-iso-cucurbic acid	Silica gel GF_{254}, 0.3 mm	Anisaldehyde: heat	Greyish blue	32
Cucurbic acid glucoside	Silica gel G, 0.25 mm	EtOH–H_2SO_4: heat (UV)	Brown (pale yellow-green)	20
	Silica gel G, 0.25 mm	Vanillin–H_2SO_4: heat	Blue-green	20
Cucurbic acid glucoside methyl ester	Silica gel G, 0.25 mm	EtOH–H_2SO_4: heat (UV)	Brown (pale yellow-green)	20
	Silica gel G, 0.25 mm	Vanillin–H_2SO_4: heat	Blue green	20

[a] AcOH–H_2SO_4–anisaldehyde (100:2:1).
[b] 5% H_2SO_4–EtOH.
[c] 5% solution of vanillin in H_2SO_4.

5.3. *High-performance liquid chromatography (HPLC)*

HPLC is suitable for the analysis and separation of plant hormones. HPLC has also been investigated intensively for the separation of jasmonates. In HPLC, UV detection is commonly used because of its sensitivity. However, jasmonates and gibberellins [42] show no significant UV absorption. For these compounds, UV detection is carried out by using an end absorption of 205–230 nm. Reversed-phase columns of medium polarity such as LiChrosorb RP-8 and Polyol RP-8 or non-polar such as LiChrosorb RP-18 and Polyol RP-18 have been used extensively [38,41,43].

5.3.1. *Free acid and methyl ester forms of jasmonates*

As shown in Tables 6 and 7, solvent systems consisting of aqueous methanol with or without a low concentration of AcOH or H_3PO_4 are commonly used for the separation of jasmonic acid [33,37,40] and its derivatives [33,37,38,40] and their methyl esters [32,37,39]. In some instances using 60% aqueous methanol containing H_3PO_4 as a solvent system, retention times longer than 100 min have been obtained [43].

5.3.2. *Conjugate jasmonates*

As shown in Table 8, jasmonates conjugated with an amino acid moiety were successfully separated by using HPLC on LiChrosorb RP-8 or Hypersil RP-8. The β-D-glucopyranosyl ester of jasmonic acid, which has not been found in the plant kingdom but has been synthesized, has also been successfully separated by using HPLC on Polyol RP-8 with the solvent system methanol–water–H_3PO_4 (40:60:0.1) [29].

5.3.3. *Enantiomers of jasmonates using diastereomeric derivatives on an achiral stationary phase*

As described above, methyl jasmonate isolated from natural sources consists of an equilibrium mixture of the 7-epimers (2-epimers) in a ratio of *ca.* 9:1. Attempts to separate the enantiomers have been made using HPLC. Previous attempts have involved conversion to the diastereomeric ketal with (−)-2,3-butanediol [44] or esters of (−)-borneol [45]. Nucleosil 10 CN and Nucleosil 100-5 columns, respectively, were used for separation. In each separation, the (+)-enantiomer eluted faster than the (−)-enantiomer with a solvent system consisting of *n*-hexane or *n*-hexane containing 13–15% of ethyl acetate (Figs. 3 and 4).

A clear separation of the isomers of the methyl ester forms of jasmonic acid and its derivatives has recently been achieved by HPLC on LiChrosorb RP-18 (7 μm) with a solvent system of methanol–water (11:9) [40]. However, free acid forms, jasmonic acid and 6-epicucurbic acid, were incompletely resolved by HPLC on

TABLE 6

HPLC OF FREE ACID FORMS OF JASMONATES

Compound	Column (length × I.D.)	Solvent system	Flow-rate (ml/min)[a]	Retention time (min)[a]	Ref.
Jasmonic acid	Zorbax ODS (150 × 4.6 mm) (40°C)	MeOH–0.01% AcOH (3:7)	–	–	33
	LiChrosorb RP-18 (250 × 10 mm)	MeOH–0.2% AcOH (11:9)	4	22.7	40
	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (11:9)	1	9.5	37
(+)-7-Iso-jasmonic acid	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (11:9)	1	9.5	37
	Polyol RP-8 (310 × 25 mm)	MeOH–H_2O–H_3PO_4 (60:40:1)	2	135–158	43
(−)-9,10-Dihydro-jasmonic acid	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (11:9)	1	13.5	37
(+)-9,10-Dihydro-7-isojasmonic acid	Polyol RP-8 (310 × 25 mm)	MeOH–H_2O–H_3PO_4 (60:40:1)	2	135–158	43
3,7-Didehydro-jasmonic acid	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (11:9)	1	7.2	37
(+)-4,5-Didehydro-7-isojasmonic acid	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (1:1)	1	9.2	38
(+)-11,12-Didehydro-7-isojasmonic acid	Polyol RP-8 (310 × 25 mm)	MeOH–H_2O–H_3PO_4 (60:40:1)	2	100–130	43
Cucurbic acid	LiChrosorb RP-18 (250 × 10 mm)	MeOH–0.2% AcOH (11:9)	4	17.3	40
6-Epicucurbic acid	LiChrosorb RP-18 (250 × 10 mm)	MeOH–0.2% AcOH (11:9)	4	22.5	40
7-Isocucurbic acid	LiChrosorb RP-18 (250 × 10 mm)	MeOH–0.2% AcOH (11:9)	4	20.5	40
6-Epi-7-iso-cucurbic acid	LiChrosorb RP-18 (250 × 10 mm)	MeOH–0.2% AcOH (11:9)	4	19.5	40
(+)-3-Oxo-2-(pentenyl)-cyclopent-1-yl-propionic acid	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (1:1)	1	11.7	36
(+)-3-Oxo-2-(pentenyl)-cyclopent-1-yl-butyric acid	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (1:1)	1	12.5	36

[a] Dashes indicate data not given.

TABLE 7

HPLC OF ESTER FORMS OF JASMONATES

Compound	Column (length × I.D.)	Solvent system	Flow-rate (ml/min)[a]	Retention time (min)[a]	Ref.
Methyl jasmonate	Nucleosil 50-5 (250 × 4.6 mm)	EtOAc–*n*-hexane (5:95)	–	–	33
	LiChrosorb RP-18 (250 × 10 mm)	MeOH–H_2O (11:9)	4	16.6	40
Ethyl (+)-7-iso-jasmonate	Polyol RP-18 (250 × 4.6 mm)	MeOH–0.1% aqueous H_3PO_4 (1:1)	1	14.5	40
Methyl cucurbate	LiChrosorb RP-18 (250 × 10 mm)	MeOH–H_2O (11:9)	4	19.5	40
Methyl 6-epicucurbate	LiChrosorb RP-18 (250 × 10 mm)	MeOH–H_2O (11.9)	4	23.0	40
Methyl 7-isocucurbate	LiChrosorb RP-18 (250 × 10 mm)	MeOH–H_2O (11:9)	4	20.3	40
Methyl 6-epi-7-iso-cucurbate	LiChrosorb RP-18 (250 × 10 mm)	MeOH–H_2O (11:9)	4	17.9	40

[a] Dashes indicate data not given.

TABLE 8

HPLC OF CONJUGATED FORMS OF JASMONATES

Compound	Column (length × I.D.)	Solvent system	Flow-rate (ml/min)	Retention time (min)[a]	Ref.
N-[(−)-Jasmonoyl]-S-tyrosine	LiChrosorb RP-8 (250 × 4.6 mm)	MeOH–0.2% aqueous AcOH (55:45)	1	–	25
	LiChrosorb RP-8 (250 × 4.6 mm)	MeOH–0.2% aqueous AcOH (45:55)	1	–	25
N-[(−)-Jasmonoyl]-S-tryptophan	LiChrosorb RP-8 (250 × 4.6 mm)	MeOH–0.2% aqueous AcOH (55:45)	1	–	26
N-[(−)-Jasmonoyl]-S-tyrosine methyl ester	LiChrosorb RP-8 (250 × 4.6 mm)	MeOH–0.2% aqueous AcOH (60:40)	1	–	25
N-[(−)-Jasmonoyl]-S-tryptophan methyl ester	LiChrosorb RP-8 (250 × 4.6 mm)	MeOH–0.2% aqueous AcOH (65:35)	1	–	25
	Hypersil RP-8 (200 × 4.6 mm)	MeOH–0.2% aqueous AcOH (40:60)	1	–	26
(−)-9,10-Dihydro-11-hydroxyjasmonic acid O-β-D-glucopyranoside	LiChrosorb RP-18 (250 × 4.6 mm)	MeOH–H_2O–AcOH (200:300:1)	0.6	7.4	24
(+)-Jasmonic acid-β-D-glucosyl ester	Polyol RP-8 (250 × 4.6 mm)	MeOH–H_2O–H_3PO_4 (40:60:0.1)	1	8.6	29
Tuberonic acid O-β-D-glucopyranoside	μBondapak (150 × 19 mm)	10% CH_3CN	9.9	–	23
	Aminex HPX-87 (300 × 7.8 mm)	0.01 *M* H_2SO_4	1	–	23
	Resolve C_{18} (150 × 3.9 mm)	30% MeOH containing 0.1% AcOH	0.45	–	23
	Novapak C_{18} (100 × 8 mm)	10% CH_3CN containing 0.1% AcOH	1	–	23
	Novapak C_{18} (100 × 8 mm)	3% THF containing 0.1% AcOH	1	–	23

[a] Dashes indicate data not given.

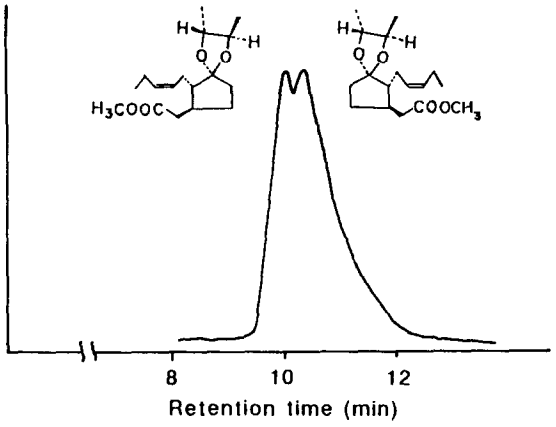


Fig. 3. HPLC of diastereomeric ketals on an analytical scale. Column, Nucleosil 10 CN (25 cm × 4 mm I.D.); mobile phase, *n*-hexane; flow-rate, 90 ml/h. Reproduced from *Agric. Biol. Chem.*, 45 (1981) 1709, by permission of the Japan Society for Bioscience, Biotechnology and Agrochemistry. Absorbance measured at 205 nm.

the same column with methanol–0.2% acetic acid (11:9) as the eluent (Tables 6 and 7) [40].

5.3.4. Enantiomers of jasmonates on a chiral stationary phase

Direct resolution using a column with a chiral stationary phase rather than the resolution of diastereomeric derivatives may be recommended for convenience. The direct separation of the enantiomers of methyl jasmonate has recently been demonstrated using a Chiralpak AS column (chiral stationary phase) [46]. As shown in Fig. 5, the optical resolution of methyl jasmonate or methyl epijasmonate (methyl 7-isojasmonate) was completely achieved by using the solvent system *n*-hexane–2-propanol (9:1) [46]. On the other hand, (±)-methyl cucurbate was not resolved by using a Chiralpak AS column but was by using a Chiralcel OF column with *n*-hexane–2-propanol as the eluent [46].

5.4. Gas chromatography (GC)

The GC of jasmonates has been extensively investigated, especially in combination with mass spectrometry for identifications. Many kinds of jasmonates can be analysed by GC after conversion into their methyl ester forms with ethereal

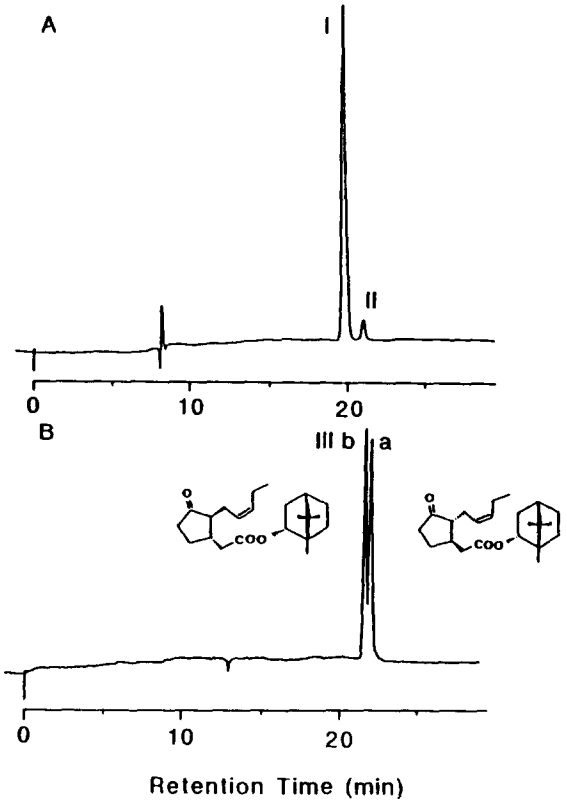


Fig. 4. HPLC of jasmonic esters (refractive index detection). (A) Methyl jasmonate (I) + methyl epijasmonate (II) (95:5). Two Nucleosil 100-5 columns (300 mm × 8 mm I.D.); mobile phase, EtOAc–hexane (15:85); flow-rate, 3 ml/min. (B) Bornyl jasmonates (IIIa:IIIb = 1:1). Three Nucleosil 100-5 columns (300 mm × 8 mm I.D.); mobile phase, EtOAc–hexane (13:87); flow-rate, 3 ml/min. The eluates were monitored with a Waters R 401 differential refractometer. Reproduced from *Agric. Biol. Chem.*, 49 (1985) 769, by permission of the Japan Society for Bioscience, Biotechnology and Agrochemistry.

diazomethane (Table 9). Because of the presence of some impurities, there is a possibility that not only may different compounds show the same retention time, but also same compound may show different retention times. Using packed columns such as SE-30 and OV-1, the retention time of methyl jasmonate differs owing to the difference in the amount of the compound injected. If a smaller amount is injected, the retention time is longer. This serious problem may be solved by using a fused-silica capillary column such as DB-1.

TABLE 9

GC OF JASMONATES

The carrier gas was nitrogen in all instances.

Compound	Column (length × I.D.)	Support	Column temperture (°C)	Flow-rate (ml/min)	Retention time (min)	Ref.
Methyl jasmonate	SE-30 (5%) (2 m × 3 mm)	Chromosorb W (80–100 mesh)	180	30	3.8	6
	EG SS-X (10%) (3 m × 4 mm)	Gas Chrom P (125–150 μm)	190	93	24.6	32
	XE-60 (3%)	–[a]	135	38	5.7	36
	OV-1 (1%) (2 m × 3 mm)	Chromosorb W (80–100 mesh)	170, 180	40	–[a]	41
	EG SS-X (10%) (4 m × 3 mm)	Gas Chrom P (125–150 μm)	170	50	38.5	43
	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	15	43
Methyl (+)-7-iso-jasmonate	EG SS-X (10%) (4 m × 3 mm)	Gas Chrom Q (100–120 mesh)	170	50	47	43
	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	17.8	43
Methyl (+)-9,10-dihydro-7-iso-jasmonate	EG SS-X (10%) (4 m × 3 mm)	Gas Chrom P (125–150 μm)	170	50	36	43
	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	15.9	43
Methyl (–)-9,10-dihydrojasmonate	EG SS-X (10%) (4 m × 3 mm)	Gas Chrom P (125–150 μm)	170	50	30.5	43
	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	13.6	43
Methyl (+)-11,12-didehydro-7-iso-jasmonate	EG SS-X (10%) (4 m × 3 mm)	Gas Chrom P (125–150 μm)	170	50	69	43
Methyl (–)-11,12-didehydrojasmonate	EG SS-X (10%) (4 m × 3 mm)	Gas Chrom P (125–150 μm)	170	50	97.5	43
Methyl (+)-cucurbate	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	21.1	43
Methyl (+)-6-epi-cucurbate	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	18.3	43
Methyl 6-epi-7-isocucurate	EG SS-X (10%) (3 m × 4 mm)	Gas Chrom P (125–150 μm)	190	93	28.2	32
Methyl 9,10-dihydrocucurbate	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	19.2	43
Methyl 9,10-dihydro-6-epi-cucurbate	OV-225 (3%) (2 m × 4 mm)	Gas Chrom Q (100–230 mesh)	150	110	16.5	43

[a] Not given.

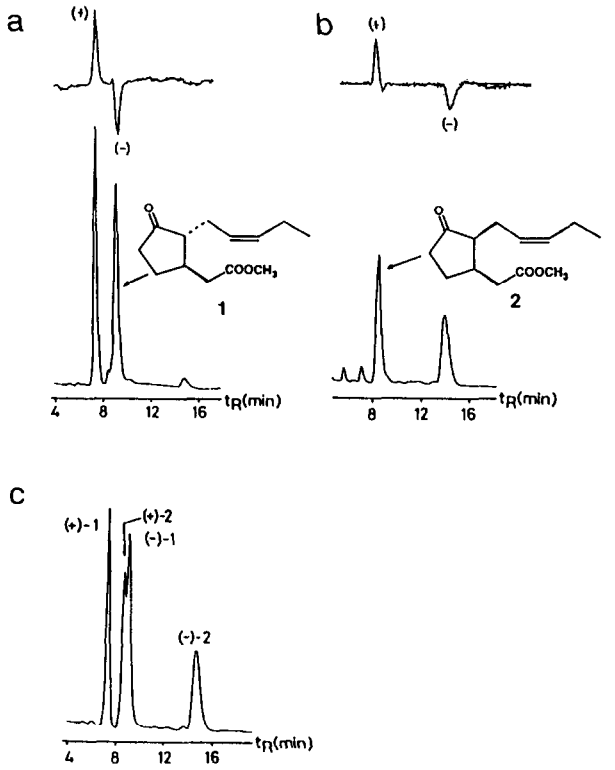


Fig. 5. (a) Chromatographic resolution of (±)-methyl jasmonate on a Chiralpak AS column (250 × 4.6 mm I.D.) (Daicel). Mobile phase, *n*-hexane–2-propanol (9:1); flow-rate, 1.0 ml/min; temperature, ambient; injection volume, 10 μl (5 μg). Upper trace, polarimetric detection (Showa Denko OR-1 employing a near-infrared LED of 780 nm); lower trace, photometric detection at 230 nm. (b) Chromatogram obtained for (±)-methyl epijasmonate. Conditions as in (a). (c) Chromatogram resulting from injection of 10 μl (5 μg each) of a mixture of (±)-methyl jasmonate and (±)-methyl epijasmonate. UV detection at 230 nm; other conditions as in (a). Reproduced from *Agric. Biol. Chem.*, 56 (1992) 1172, by permission of the Japan Society for Bioscience, Biotechnology and Agrochemistry.

The column temperature in analyses using packed columns is around 180°C, as in the analysis of fatty acid methyl esters [47]. The separation of the isomers of the methyl esters was performed by using a cross-linked methylsilicone fused-silica column with a temperature programme such as 50°C isothermal for 1 min, increased to 140°C at 25°C/min, held at 140°C for 1 min and then increased to 160°C at 2.5°C/min.

6. QUALITATIVE AND QUANTITATIVE ANALYSES OF JASMONATES BY USING COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS) AND WITH SELECTED-ION MONITORING (GC–SIM)

Methyl esters of jasmonates can be easily separated and analysed by GC, and the identification of jasmonates can be successfully performed by using combined GC–MS. This method is convenient and reliable for the identification of jasmonates. In this method, a fused-silica capillary column is commonly used because of its good separation properties. Mass spectral data for methyl ester forms of jasmonates are summarized in Table 10.

Combined GC–MS with selected-ion monitoring (GC–SIM) is used not only as an identification method but also in quantitative analysis. The GC–SIM profile of methyl jasmonate is shown in Fig. 6. For the determination of methyl jasmonate using [9,10-^{2}H] jasmonic acid as an internal standard, prominent peaks at *m/z* 224 and 226, which are the molecular ion peaks of methyl jasmonate and the deuterium-labelled compound, respectively, are monitored [48]. The level of jasmonic acid in the sample was de-

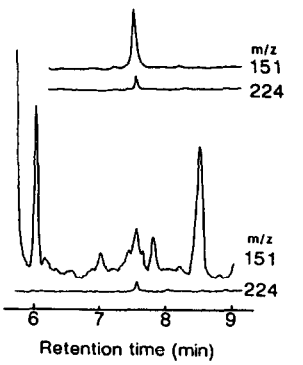


Fig. 6. GC–SIM profile of an authentic sample of methyl jasmonate (top) and the methyl ester form of an unknown material in the jasmonic acid fraction of *Euglena gracilis* Z (bottom). JEOL DX-300 mass spectrometer combined with a gas chromatograph (160°C isothermal, 70 eV, 300 μA). A DB-1 fused-silica capillary column (30 m × 0.254 mm I.D.) was used. The carrier gas was helium and the splitting ratio was 12:1. Reproduced from *Agric. Biol. Chem.*, 55 (1991) 275, by permission of the Japan Society for Bioscience, Biotechnology and Agrochemistry.

TABLE 10

MASS SPECTRAL DATA FOR THE METHYL ESTERS OF JASMONATES

Compound	*m/z* (relative intensity, %)	Ref.
Jasmonic acid	224(M^+, 37), 206(13), 193(17), 177(14), 156(24), 151(50), 135(20), 133(20), 121(17), 109(34), 95(44), 83(100)	6
(−)-9,10-Dihydro-jasmonic acid	226(M^+, 3), 195(3), 156(38), 153(34), 83(100)	49
(+)-9,10-Dihydro-7-isojasmonic acid	226(M^+, 3), 195(3), 156(38), 153(34), 83(100)	49
4,5-Didehydro-7-iso-jasmonic acid	222(M^+, 21), 193(17), 191(11), 167(15), 154(81), 149(18), 133(21), 119(16), 107(21), 95(100)	38
(+)-3-Oxo-2-(2-pentenyl)cyclopent-1-yl-propionic acid	238(M^+, 24), 220(16), 207(12), 191(18), 170(21), 164(8), 165(7), 151(75), 133(13), 121(9), 109(40), 97(67), 83(100)	38
(+)-3-Oxo-2--(2-pentenyl)cyclopent--1-yl-butylric acid	252(M^+, 15), 234(12), 221(5), 196(10), 184(12), 151(61), 133(27), 124(13), 109(27), 95(37), 83(100)	38
(+)-7-Isojasmonic acid[a]	238(M^+, 23), 220(6), 209(4), 193(17), 191(12), 170(16), 151(43), 133(23), 109(34), 95(43), 93(39), 83(100), 79(35)	38
(−)-9,10-Dihydro-11-hydroxyjasmonic acid[b]	242(M^+, 5), 224(1), 211(4), 209(5), 198(3), 182(3), 169(20), 156(81), 151(35), 137(10), 125(22), 109(29), 96(27), 83(100)	24
Cucurbic acid	226(M^+, 1), 208(9), 195(2), 165(7), 156(13), 153(73), 152(32), 139(11), 134(33), 119(16), 83(100), 79(66), 74(26)	40
6-Epicucurbic acid	226(M^+, 3), 208(12), 195(16), 165(17), 156(2), 153(33), 152(63), 139(42), 134(96), 119(61), 83(48), 79(100), 74(28)	40
6-Epi-7-isocucurbic acid	226(M^+, 2), 208(12), 165(7), 153(7), 152(19), 139(24), 134(100), 119(30), 83(38), 79(59), 74(22)	40
N-[(−)-Jasmonoyl]-S-tryptophan	410(M^+, 4), 378(4), 201(41), 170(4), 159(4), 143(5), 130(100), 117(3)	26
N-[(−)-Jasmonoyl]-S-tyrosine	378(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)	25
N-[(+)-Cucurbinoyl]-S-tryptophan	412(M^+, 6), 394(2), 201(74), 170(6), 159(7), 143(8), 130(100), 117(3)	26

[a] Ethyl ester form.
[b] Mixture with (−)-9,10-dihydro-12-hydroxyjasmonic acid.

termined from the ratio of the *m/z* 224 and 226 peak areas.

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