Distribution and Stereochemistry of Hydroxycinnamoylmalic Acids and of Free Malic Acids in Papaveraceae and Fumariaceae

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The distribution of hydroxycinnamoylmalic acids (HCM acids) in 25 species and 2 subspecies of Papaveraceae and Fumariaceae representing 14 genera was investigated by HPLC and subsequent diode array detection (DAD). In case of similar chromatographic and UV-spectrometric behavior of HCM acids and other hydroxycinnamic acid derivatives liquid chromatography-thermospray/mass spectrometry (LC-TSP/MS) was used for positive identification. Ten of the species were found to contain caffeoyl-, *p*-coumaroyl-, and feruloylmalic acid whereas *Chelidonium majus* only showed the presence of caffeoylmalic acid. Sinapoylmalic acid could not be detected. The quantitative determination by HPLC at 330 nm yielded the highest content of caffeoylmalic acid for *Fumaria officinalis* and *F. capreolata*, and the lowest for *Chelidonium majus*. Isolated HCM acids showed positive optical rotation for members of the Papaveraceae and negative values for members of the Fumariaceae. This points to an esterification of hydroxycinnamic acids with (–)-L-malic acid for the Papaveraceae and with (+)-D-malic acid for the Fumariaceae. For plants containing HCM acids the contents of free L- and D-malic acid were determined enzymatically. Yields of L-malic acid were higher for Papaveraceae than for Fumariaceae; D-malic acid was detectable only in traces for all investigated species. The Fumariaceae showed a ca. 1:1 relationship between free L-malate and esterified D-malate. This suggests an enzymatic racemization of the original L-malate and a subsequent esterification of the intermediate D-malate with hydroxycinnamic acids.

Introduction

Higher plants often contain a large number of benzoic and cinnamic acid derivatives with different physiological effects (Veit and Gumbinger, 1993) and taxonomic significance (Bate-Smith, 1962; Mølgaard and Ravn, 1988; Boucherau *et al.*, 1991; Veit *et al.*, 1992). This group of chemotaxonomic markers includes naturally occurring hydroxycinnamic acid esters with aliphatic hydroxycarboxylic acids as alcohol moiety such as esters of quinic acid (Moeller and Herrmann, 1983; Adzet and Puigmacia, 1985), tartraric acid (Veit and Strack, 1991; Soicke *et al.* 1988), tartronic acid (Terencio *et al.*, 1993) or malic acid. HCM acids were first isolated as 2-O-caffeoyl-L-malate from *Phaseolus vulgaris* [(phaselic acid, (Scarpati and Oriente, 1960)] and *Trifolium pratense* (Yoshihara *et al.*, 1977), as 2-O-(*p*-coumaroyl)-L-malate, 2-O-feruloyl-L-malate (Nielsen *et al.*, 1984), and as 2-O-sinapoyl-L-malate from *Raphanus sativus* (Linscheid *et al.* 1980). Further occurrence of HCM acids without values for optical rotation has been reported for some Fabacean vegetables (Winter and Herrmann, 1986) and for *Parietaria officinalis* and *Urtica dioica*, both Urticaceae (Budzianowski, 1990; 1991).

The first report on esterification of caffeic, *p*-coumaric, and ferulic acid with (+)-D-malic acid dates back to 1993, when (–)-caffeoyl-, (–)-*p*-coumaroyl-, and (–)-feruloyl-D-malic acid were

Abbreviations: DAD, diode array detection; HPLC, high performance liquid chromatography; LC-TSP/MS, liquid chromatography-thermospray/mass spectrometry; HCM acids, hydroxycinnamoylmalic acids; HCA, hydroxycinnamic acid; HCAD, hydroxycinnamic acid derivatives.

Part of the projected Ph.D. thesis of S. C. Boegge.

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isolated from the upper parts of *Fumaria officinalis*, Fumariaceae (Hahn and Nahrstedt, 1993a). Two years earlier (+)-caffeoyl-L-malic acid was isolated from *Chelidonium majus*, Papaveraceae (Hahn and Nahrstedt, 1991). These results prompted a study on the distribution of HCM acids in Papaveraceae and Fumariaceae. Not only the occurrence but especially the optical rotation were investigated in order to reveal the significance as taxonomic feature. Although HPLC-screening methods with UV- or DAD for hydroxycinnamic acid derivatives are elegant and well established (Winter and Herrmann, 1984; Brandl and Herrmann, 1983; Torres *et al.*, 1987), sometimes uncertainties arise on the identification of substances with similar chromatographic behavior and comparable UV-maxima. Wolfender *et al.* (1994) have reported on the problem of complex plant extracts and identification of substances by only chromatographic and UV-spectroscopic methods and proposed LC-MS-methods as suitable analytical tool to get definite information about the composition of extracts and distribution of compounds. Thus we developed an LC-TSP/MS-method to confirm the occurrence and distribution of hydroxycinnamic acid malate esters in the crude plant extracts of Fumariaceae and Papaveraceae.

Further attention was turned towards the occurrence of free L- and D-malic acid in the aqueous plant extracts. Although L-malic acid is described as the naturally occurring enantiomer, many determinations of malic acid are lacking data about stereochemistry (Ruhl *et al*, 1985; Kringstadt and Franck Backe, 1977). The frequency of D-malate occurrence, thus, might be higher than exemplified so far. The enzymes of HCA conjugation are recruited from two groups, glucosyltransferases and acyltransferases. The pathway of several HCA esters such as rosmarinic acid (Peteren and Alfermann, 1988), caffeoylisocitric acid (Strack *et al.*, 1987) and some HCA-glucuronates and HCA-galactarates (Strack *et al.*, 1987) is known as well as formation of HCA-L-malate esters. For esterification of intermediately liberated sinapic acid in red-radish seedlings *via* transiently accumulating 1-O-sinapoyl-D-glucose the transacylase uses L-malate for formation of 2-O-sinapoyl-L-malate (Strack, 1982). No reference was available about an esterfication of HCA with the D-enantiomer.

Materials and Methods

Instrumentation

Extraction was performed with an Ultra Turrax® T25 (Janke & Kunkel, Staufen, Germany) at 13500 rpm, centrifugation with an UJ III E apparatus (Heraeus-Christ, Osterode, Germany); a Delta I or Delta I-5 system (Heraeus-Christ, Osterode, Germany) was used for freeze-drying; optical rotations were measured on a Perkin-Elmer 241 apparatus, ^{1}H NMR spectra were measured at 199.9 MHz on a Varian Gemini 200 spectrometer (methanol as internal standard).

Plant material

Aerial parts of flowering plants of *Corydalis cava, Dicentra spectabilis, Papaver bracteatum*, and *Hypecoum procumbens* were collected in Bad Marienberg (April 1993), Münster (May 1993), Münster-Hiltrup (May 1994), all Germany, and near Ankara, Turkey (May 1994). All other plants were cultivated in the Experimental Garden of the Institute of Pharmaceutical Biology and Phytochemistry, Münster. The aerial parts were harvested during the flowering stage, ground in liquid nitrogen, and freeze-dried. Voucher specimens are deposited at the Institute (PBMS 16, 81, 82, 91–105, 112–118).

Isolation

For isolation of metabolites, freeze-dried and powdered plant material (about 15 g each) was extracted using an Ultra-Turrax® homogenizer for several minutes under cooling by an ice water-bath. Extraction was done three times with 30 ml methanol/water (3:2 v/v) each. After centrifugation (5000 rpm for 5 min) the combined supernatants were evaporated under reduced pressure at 40 °C. The aqueous residue was left at +4 °C for 12 hours to allow the chlorophyll to precipitate. After filtration the extract was adjusted to pH 2 (trifluoroacetic acid) and subjected to an exhaustive liquid-liquid extraction with diethylether. The freeze-dried diethylether solubles were fractionated by Sephadex LH-20 chromatography (15×300 mm, Pharmacia, Uppsala, Sweden) with methanol/water (1:1 v/v) as eluent, flow-rate 14 ml/h, fraction-size 3.5 ml. Control of the fractions was done by TLC and subsequent diphenyl-

boryloxyethylamine (1% in MeOH)/UV_{365} detection. Fraction 81–105 ml usually contained feruloylmalic, fraction 105–126 ml *p*-coumaroylmalic, and fraction 126–151 ml caffeoylmalic acid. Final purification was carried out with preparative HPLC (system 3 and 4), identity of the isolates was checked by ^1H NMR or TLC in case of poor yields, optical rotation was measured in water at 20 °C.

Determination of free hydroxycinnamic acids

3.0 g freeze dried and powdered herb were extracted as described above. After filtration of the chlorophyll the solution was adjusted to pH 4 (TFA), mixed with Röhm enzyme (crude enzyme preparation, EL-177; Röhm, Darmstadt, Germany), and left for 15 h at 45 °C for quantiative hydrolysis. After filtration the solution was diluted to 10.0 ml and aliquots of 5 µl were analysed by HPLC (system 1). For determination of recovery, 5.0 mg of each caffeic, *p*-coumaric, ferulic and sinapic acid (all Fluka, CH Buchs) were dissolved in MeOH/H_2O (3:2) and treated as described above. Contents of free hydroxycinnamic acids were calculated by calibration equation of each hydrocinnamic acid.

Quantification of hydroxycinnamic acid derivatives (HCAD total) and HCM acids

For quantitative determination, 3.0 g freeze-dried and powdered material were extracted as described above. After filtration of chlorophyll the clear solution was diluted with water to 10.0 ml, and either analysed within the next day or stored at −20 °C until determination. For HPLC-DAD-analysis (system 2) aliquots of 5 µl were injected. LC-TSP/MS-analysis needed a dilution of the aliquots with water (1:1 or 1:2) before determination. The HCM acid content was calculated by the calibration equation of each HCM acid. The content of total hydroxycinnamic acid derivatives (HCAD total) bases on the calibration equation of caffeoylmalic acid.

Preparation of standard solutions and calibration curves

15.0 mg of caffeic, *p*-coumaric, ferulic and sinapic acid each were dissolved in MeCN/H_2O with 5% phosphoric acid (2:3) and diluted 1:1, 1:2 and 1:3 with the same solvent. Aliquots of 5 µl of all dilutions were analysed three times (system 1). Correlation coefficients were 0.9986 for caffeic, 0.9976 for *p*-coumaric, 0.9972 for ferulic, and 0.9988 for sinapic acid. Recovery rates were 88% for caffeic, 91% for *p*-coumaric, 96% for ferulic, and 92% for sinapic acid. 3.83 mg of caffeoylmalic (CM) and 3.87 mg of feruloylmalic acid (FM) were dissolved in 10.0 ml water. Appropriate aliquots of this standard solution were diluted with water to obtain a series of four solutions containing 0.383 to 0.096 mg/ml. All dilutions were analysed in triplicate (system 2). Calibration curves were based on peak area ratio by means of linear regression (n = 12). The correlation equations are $Y = 0.030\ X - 5.037$ (correlation coefficient, $r = 0.9987$) for caffeoylmalic and $Y = 0.013\ X - 1.784$ (correlation coefficient, $r = 0.9976$) for FM. Since *p*-coumaroylmalic acid (p-CM) could not be isolated in amount sufficient for a calibration curve, contents were calculated using the calibration equation of FM with correction factor 0.67 (abs. coeff. p-$CM_{330\ nm}$ divided by abs. coeff. $FM_{330\ nm}$: 6988/10471 = 0.67)

TLC

TLC was performed on silica gel (pre-coated aluminium sheets, layer thickness 0.2 mm, Merck, Darmstadt, Germany) with EtOAc/MeOH/H_2O/HCOOH, 100:4:8:5 (system1) and EtOAc/HOAc/HCOOH/H_2O, 100:11:11:27 (system 2) as mobile phases. R_f values are: 0.74 (system 1) and 0.87 (system 2) for caffeoylmalic, 0.83/0.88 for *p*-coumaroylmalic, and 0.81 and 0.91 for feruloylmalic acid.

Analytical HPLC-DAD

Analytical HPLC-DAD was carried out with a Waters 600 pump, a Waters 990 PDA detector with Waters 990 plotter, and a Rheodyne 8125 injector with 5 µl sample loop. The column used was a ProSep® C18 cartridge, 5 µm, 150×4.0 mm ID with cartridge holder (Latek, Eppelheim, Germany).

System 1: 0–17 min 9% MeCN/H_2O with 0.5% phosphoric acid isocratic, 17–25 min 9 to 19% MeCN, 25–35 min 19 to 40% MeCN, 35–40 min 40 to 60% MeCN, 40–50 min 60 to 100% MeCN, flow 1 ml/min; detection at 320 nm; temp. set at 35 °C. Retention times are 8.9 min for caffeic, 17.3

min for *p*-coumaric, 23.6 min for ferulic, and 25.3 min for sinapic acid. System 2: 0–20 min 0 to 7% MeCN/H_2O with 1% HOAc, 20–35 min 7 to 25% MeCN, 35–40 min 25 to 50% MeCN, 40–45 min 50 to 100% MeCN, flow 2 ml/min; detection at 237, 260, 330, and 360 nm. Retention times are 22 min for caffeoylmalic, 25.2 min for *p*-coumaroylmalic, and 28.1 min for feruloylmalic acid.

Preparative HPLC

Preparative HPLC was carried out with a Waters 600 pump, a Waters 490 Programmable Multiwavelength Detector, and a Rheodyne 7125 injector with 140 µl sample loop. Column: Latek RP18, 8 µm, 250×20 mm ID, with guard column, RP18, 5 µm, 30×20 mm ID. System 3 for purification of caffeoylmalic acid: 15% MeCN/H_2O with 0.1% TFA isocratic; flow 12 ml/min; detection at 315 and 330 nm. System 4 for purification of *p*-coumaroylmalic and feruloylmalic acid: 20% MeCN/H_2O with 0.1% TFA isocratic; flow 12 ml/min; detection at 315 and 330 nm.

LC-TSP/MS

For chromatography, two LDC/Milton-Roy constaMetric III dual pistonpumps equipped with a pulse dampener (LDC, Darmstadt, Germany), a Rheodyne 7125 injector with 20 µl sample loop, and a Nucleosil 120 RP18 column, 5 µm, 100×2 mm ID, with guard column, 7×2 mm ID (Knauer,Berlin, Germany) were applied. The gradient was formed with a D/A converter, controlled by a self-written software (ISAS, Dortmund, Germany). The make-up flow consisted of MeOH/H_2O (80:20 v/v) with 0.1 mol/l ammonium acetate; the flow rate was kept at 0.7 ml/min, produced by an ISCO 2600 syringe pump (Colora, Lorch, Germany), and introduced *via* a stainless-steel tee. Chromatographic conditions (system 5): solvent A H_2O with 1% HOAc, solvent B H_2O with 1% HOAc/MeOH (65:35; 0.5 ml/min); gradient table: 0–10 min flow solvent A 0.37 ml/min, flow solvent B 0.13 ml/min isocratic; 10–15 min flow A 0.13 ml/min, flow B 0.37 ml/min linear; determination duration 35 min. Mass spectrometry was carried out using a Finnigan MAT 4500 and a Finnigan MAT TSP-interface (Finnigan MAT, San José, CA, USA) at a source temperature of 250° C; the vaporizer was set at 123 °C. The pumps were two turbo pumps 330 l/s (Pfeiffer, Asslar, Germany) with an Edwards E2M12 (m^3/h) roughing pump (Edwards, Marburg, Germany); TSP pump was a Busch 025 rotary pump. Negative ions were recorded. For data acquisition, a model 802 analog interface and Vector/One software (Teknivent, Missouri, USA) was used. Spectra were acquired as peak profile data and further evaluated using "MSGRAPH" software (Hau and Linscheid, 1993). HCM acids gave as main signals $[M-H]^-$ parent ions of *m/z* 295, 279 and 309 for caffeoyl-, *p*-coumaroyl-, and feruloylmalic acid respectively, as well as *m/z* of 179, 163 and 193 for the caffeoyl, *p*-coumaroyl and feruloyl moiety.

Enzymatic determination of free L- *and* D-*malic acid*

2 g freeze dried and powdered herb were extracted with water using an Ultra Turrax® homogenizer for 5 min at 9500 rpm. After centrifugation at 5000 rpm for 5 min and filtration the extract required a decolorization and absorption of tannins by addition of 200 mg polyamide (Polyamide CC 6, 0.07 mm, Macherey & Nagel, Düren, Germany). The supernatant of the centrifuged solution was then evaporated at 40 °C under reduced pressure and diluted with water to 5.0 or 10.0 ml. This stock solution was diluted 1:10 and applied to the analysis of L-malic acid. For determination of D-malic acid, the stock solution was further purified by a small polyamide column (20×5 mm) in order to obtain a stronger decolorization because of very low absorption differences (see instructions by Boehringer). Dilutions of 3:10 were applied to analysis. Enzymatic determination was carried out with test combination specific for either L-malic or D-malic acid following the instructions by Boehringer (Boehringer Mannheim, Cat. No. 139068 and 1215558). Formed NADH was measured at 340 nm. Determination was done twice with a quantitative re-start for one reaction with L- or D-malic acid standard solution. Determination limit was 0.018 g/l D-malic acid in sample solution.

Results and Discussion

Quantitative determination of the free hydroxycinnamic acids after enzymatic hydrolysis of the extracts confirms caffeic acid as main hydroxy-

cinnamic acid in all species. The yields of the free HCAs in the total plant extracts are given in Table I for 4 species of Papaveraceae and 7 of the Fumariaceae including two formae. As remarkable items there are a sequence of free hydroxycinnamic acid content following CA > FA > pCA > SA (except for *D. spectabilis* with pCA > FA) and a higher content of total hydroxycinnamic acid derivatives than of the summed hydroxycinnamic acid moieties for all species.

Quantitative determination of the HCM acids in the total plant extract was carried out by HPLC at 330 nm. Identity and peak purity were confirmed by DAD and LC-TSP/MS. Table II presents the amount of total HCM acids as well as the contents of each HCM acid in 4 species of Papaveraceae and 7 Fumariaceae including 2 formae. The results show a higher total accumulation (0.41–1.33%) for members of the Fumariaceae than for Papaveraceae (0.06–0.39%). Except for *Dicentra spectabilis* the main HCM acid for all species is the caffeoyl derivative followed by feruloyl- and *p*-coumaroylmalic acid. Investigations of *C. lutea*

Table II. Contents of total hydroxycinnamoylmalic acids (ΣHCM acids) and of individual HCM acids in 11 species of Papaveraceae and Fumariaceae. Results are given in percent of freeze-dried plant material. CM, caffeoylmalic acid; p-CM, *p*-coumaroylmalic acid; FM, feruloylmalic acid.

Plant	Harvest	Total HCM acids	CM (%)	p-CM (%)	FM (%)
Papaveraceae					
Glaucium flavum	06–93	0.34	0.12	0.08	0.14
Glaucium corniculatum	08–93	0.39	0.21	0.02	0.16
Chelidonium majus	05–92	0.06	0.06	(–)	(–)
Macleaya microcarpa	10–93	0.17	0.15	(–)	0.02
Fumariaceae					
Adlumia fungosa	07–94	0.83	0.69	0.03	0.11
Fumaria officinalis	06–92	1.33	1.04	0.07	0.22
Fumaria capreolata	06–92	1.22	1.02	0.08	0.12
Dicentra spectabilis	05–93	0.41	0.16	0.20	0.05
Corydalis cava	04–93	1.05	0.75	0.04	0.26
Corydalis cava f. *albiflora*	04–93	0.57	0.34	0.08	0.15
Corydalis lutea	07–93	0.99	0.77	0.04	0.18
Corydalis lutea	08–93	0.72	0.54	0.03	0.15
Corydalis lutea	10–93	0.86	0.66	0.04	0.16
Corydalis sempervirens	06–93	0.65	0.42	0.03	0.20

Table I. Contents of total hydroxycinnamic acid derivatives (HCAD total, see Materials and Methods) and free hydroxycinnamic acids after enzymatic hydrolysis based on the calibration curve of caffeoylmalic acid and of the calibration curves of each hydroxycinnamic acid (HCA). Results are given in percent of freeze-dried plant material. CA, caffeic acid; p-CA, *p*-coumaric acid; FA, ferulic acid; SA, sinapic acid.

Plant	Harvest	0 HCAD total (%)	1 CA (%)	2 p-CA (%)	3 FA (%)	4 SA (%)	Σ 1–4 HCA moiety total
Papaveraceae							
Glaucium flavum Crantz	06–93	0.46	0.10	0.08	0.08	0.02	0.28
Glaucium corniculatum (L.) Pers.	06–93	0.65	0.15	0.02	0.03	–	0.47
Chelidonium majus L.	05–92	1.20	0.53	0.06	0.02	–	0.61
Macleaya microcarpa (Maxim.) Fedde	10–93	0.39	0.10	0.03	0.15	<0.01	0.29
Fumariaceae							
Adlumia fungosa (Ait.) Greene	07–94	1.14	0.45	0.06	0.09	0.02	0.62
Fumaria officinalis L.	06–92	1.23	0.32	0.04	0.04	–	0.40
Fumaria capreolata L.	06–92	1.22	0.41	0.08	0.04	0.02	0.55
Dicentra spectabilis (L.) Lem.	05–93	0.67	0.13	0.12	0.04	–	0.29
Corydalis cava (L.) Schweigg. et Koerte	04–93	1.32	0.32	0.06	0.09	<0.01	0.48
Corydalis cava (L.) Schweigg. et Koerte f. *albiflora*	04–93	0.73	0.35	0.06	0.11	0.02	0.54
Corydalis lutea L.	07–93	0.74	0.32	0.02	0.02	0.01	0.37
Corydalis sempervirens (L.) Pers.	06–93	0.98	0.15	0.03	0.15	<0.01	0.34

during flowering stage for three months successively gave no remarkable differences in total or individual HCM content. Root material of *C. sempervierens* yielded no HCM acids at all.

In case of higher amounts of total HCAD than of ΣHCM acids the species contain other hydroxycinnamic acid derivatives some of which have been elucidated yet. In case of conformity the main HCAD are HCM acids. Papaveraceae and Fumariaceae do not show remarkable differences concerning contents of free HCA and HCM acids.

Previous work on HCM acids of *Chelidonium majus* and *Fumaria officinalis* has shown inverse optical rotation of the caffeoylmalic acids (Hahn and Nahrstedt, 1991; 1993a): negative values were obtained for caffeoylmalic-, *p*-coumaroylmalic and feruloylmalic acid of *F. officinalis*, positive values for caffeoylmalic acid of *C. majus*. Values for optical rotation of HCM acids of the investigated species are given in Table III. Identity of the isolated compounds was finally confirmed by ^{1}H NMR in comparison with Hahn and Nahrstedt (1993a; 1993b). Values for the optical rotation of (+)-caffeoylmalic acid are in good accordance with (Yoshihara *et al.*, 1977; Linscheid *et al.*, 1980; Hahn and Nahrstedt, 1993a) just as well as values for (−)-*p*-coumaroylmalic and (−)-feruloylmalic acid correspond with the literature (Hahn and Nahrstedt, 1993b). Optical rotation of (+)-feruloylmalic acid is described here for the first time.

As a result the HCM acids isolated so far from Papaveraceae show positive values for optical rotation whereas those isolated from members of Fumariaceae show negative values. Although the number of investigated species was limited we suggest the optical rotation of the HCM acids as a distinctive taxonomic feature between these closely related families. Further, the HCM acids with positive optical rotation seem to be accumulated only in the subfamily Chelidonioideae of the Papaveraceae. HCM acids with negative optical rotation only occur in the Fumarioideae of the Fumariaceae (for taxonomy see Kadereit, 1993; Lidén, 1993). Besides the optical rotation of the HCM acids the inverse rotation for the corresponding free malic acid should be noted. Papaveraceae apparently use (−)-L-malic acid for esterification with hydroxycinnamic acids whereas Fumariaceae seem to use the rarely occurring (+)-D-malic acid for their esterification.

Enzymatic determination of free L- and D-malic acids in the HCM acids containing species yielded higher contents of L-malic acid for Papaveraceae than for Fumariaceae (Table IV). Free D-malic acid was detectable only in traces for all species. Comparison of enzymatically determined free ma-

Table III. Values for optical rotation (solvent H_2O) of the isolated HCM acids. Components in blank cells could not be isolated because of low yield or recurrent decomposition during purification. CM, caffeoylmalic acid; p-CM, *p*-coumaroylmalic acid; FM, feruloylmalic acid. Identity of the marked compounds (*) was confirmed by ^{1}H NMR. Components marked with (−) could not be detected.

Plant	$[\alpha]_D^{20}$ CM	$[\alpha]_D^{20}$ p-CM	$[\alpha]_D^{20}$ FM
Papaveraceae			
Glaucium flavum	+29.9 (*c* 1.86)*		+15.0 (*c* 0.40)
Glaucium corniculatum	+29.2 (*c* 4.10)*		+14.0 (*c* 1.25)*
Chelidonium majus[a]	+31.5 (*c* 1.36)*	(−)	(−)
Macleaya microcarpa	+30.4 (*c* 0.84)*		
Fumariaceae			
Adlumia fungosa	−26.2 (*c* 2.42)*		
Fumaria officinalis[a]	−24.4 (*c* 0.87)*	−6.7 (*c* 0.45)*	−15.3 (*c* 1.01)*
Fumaria capreolata	−23.6 (*c* 21.0)*		
Dicentra spectabilis	−26.7 (*c* 1.124)*	−7.5 (*c* 5.0)*	
Corydalis cava	−25.0 (*c* 0.94)*		−19.2 (*c* 0.86)
Corydalis lutea	−26.9 (*c* 0.86)*		−14.0 (*c* 0.25)
Corydalis sempervirens	−25.2 (*c* 3.0)*		

[a] According to Hahn and Nahrstedt, 1993a; 1993b.

Table IV. Contents of free and esterified (−)-L-malic acid and (+)-D-malic acid in aqueous extracts of freeze-dried plant material. −, Content lower than determination limit (0.018 g/l D-malic acid); determination was checked by quantitative re-start of the reaction with D-malic acid standard solution. (−), Content lower than determination limit (0.018 g/l D-malic acid); determination could not be assured by a quantitative re-start of the reaction with standard solution because of unknown interferences between sample solution and enzyme preparation.

Plant	Harvest	Content of free (−)-L-malic acid (g/100 g)	Content of free (+)-D-malic acid (g/100 g)	Content of esterified (−)-L-malic acid (%)[a]	Content of esterified (+)-D-malic acid (%)[a]
Papaveraceae					
Glaucium flavum	06–93	1.69	(−)	0.13	
Glaucium corniculatum	08–93	1.71	−	0.15	
Chelidonium majus	05–92	1.05	−	0.02	
Macleaya microcarpa	10–93	4.43	−	0.07	
Fumariaceae					
Adlumia fungosa	08–94	0.58	(−)		0.32
Fumaria officinalis	06–92	0.40	−		0.52
Fumaria capreolata	06–92	0.30	(−)		0.48
Dicentra spectabilis	07–94	0.23	−		0.17 (05–93)
Corydalis cava	04–93	0.32	(−)		0.41
Corydalis lutea	10–93	0.39	(−)		0.34
Corydalis sempervirens	06–93	0.25	(−)		0.25

[a] Determination on the basis of HPLC quantification of each HCM acid at 330 nm. The alcohol moiety amounts to 39.2% of CM, 41.4% of p-CM, and 37.4% of FM. There was no yield for components in blank cells.

lates with HPLC determined esterified malates gave a ca. 1:1 relationship between the free L-malic acid and esterfied D-malate for the Fumariaceae. No relationship between free and esterfied L-malate for Papaveraceae could be found. On the basis of the present data we conclude that the biogenetically formed enantiomer of malic acid is the L-enantiomer wich is either directly used for esterification with activated HCA as presumed for the Papaveraceae or which becomes racemized prior to the esterification step as presumed for the Fumariaceae. Enzymatic racemization of an L-enantiomer usually causes a ca. 1:1 mixture of L- and D-form of which the D-malate in Fumariaceae might be removed by subsequent esterification. Details about the esterification step and its stereochemistry as well as investigations about the enzymes involved should find further attention.

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