N-(Jasmonoyl)tyrosine-Derived Compounds from Flowers of Broad Beans (*Vicia faba*)

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Two new amide-linked conjugates of jasmonic acid, *N*-[(3*R*,7*R*)-(−)-jasmonoyl]-(*S*)-dopa (**3**) and *N*-[(3*R*,7*R*)-(−)-jasmonoyl]-dopamine (**5**), were isolated in addition to the known compound *N*-[(3*R*,7*R*)-(−)-jasmonoyl]-(*S*)-tyrosine (**2**) from the methanolic extract of flowers of broad bean (*Vicia faba*). Their structures were proposed on the basis of spectroscopic data (LC-MS/MS) and chromatographic properties on reversed and chiral phases and confirmed by partial syntheses. Furthermore, tyrosine conjugates of two cucurbic acid isomers (**7**, **8**) were detected and characterized by LC-MS. Crude enzyme preparations from flowers of *V. faba* hydroxylated both (±)-**2** and *N*-[(3*R*,7*R*/3*S*,7*S*)-(−)-jasmonoyl]tyramine [(±)-**4**] to (±)-**3** and (±)-**5**, respectively, suggesting a possible biosynthetic relationship. In addition, a commercial tyrosinase (mushroom) and a tyrosinase-containing extract from hairy roots of red beet exhibited the same catalytic properties, but with different substrate specificities. The conjugates (±)-**2**, (±)-**3**, (±)-**4**, and (±)-**5** exhibited in a bioassay low activity to elicit alkaloid formation in comparison to free (±)-jasmonic acid [(±)-**1**].

Jasmonic acid (JA) and methyl jasmonate are the most common representatives of jasmonates, a class of plant hormones derived from linolenic acid via the oxylipin pathway.[1] Structurally related compounds such as cucurbic acid (CA) including its isomers and hydroxylated JAs are known to be native compounds in higher plants.[2,3] Previous analyses of broad beans (*Vicia faba*) revealed the occurrence of jasmonic acid derivatives. From fruits and flowers of *V. faba*, JA conjugated to aromatic amino acids such as tyrosine[4] and tryptophan[5] were isolated. Furthermore, JA conjugates derived from the aliphatic amino acids isoleucine, leucine, and valine were identified in barley leaves.[6a,b] Both the isoleucine conjugate of JA and that of 7-isocucurbic acid were found to be native compounds in pollen of mountain pine (*Pinus mugo*).[7] The occurrence of (9,10-dihydrojasmonoyl)tryptophan in *Asparagus officinalis* has been published recently.[8] The JA conjugate with tyramine, a biogenic amine, was isolated from pollen of *Petunia hybrida*.[9] Although compounds of this type could be detected as native jasmonates in several plants, only a few investigations on their physiological roles and biological functions are known. The treatment of barley leaves with *N*-(jasmonoyl)-(*S*)-isoleucine induced formation of specific proteins indicating that such conjugates are biologically active as signaling molecules.[6b,10] Elicitation of phytoalexin formation after application of JA and JA amino acid conjugates to rice leaves has been described.[11a,b]

As (*S*)-3-(3,4-dihydroxyphenyl)alanine (dopa) is one of the predominant amino acids in broad bean hypocotyls,[12] pods,[13] and flowers,[14] the question arose whether this amino acid also occurred conjugated with jasmonic acid. Therefore, a reinvestigation of broad bean flowers concerning JA-derived compounds was performed. Dopa is formed in plants from tyrosine by the hydroxylating activity of tyrosinase.[15,16] When dopa-containing plants are injured, the wounded parts turn brown or black, due to the oxidation of dopa to melanin by polyphenol oxidases.[17] This melanin is also responsible for the flower coloration (black wing spots) of broad beans.[14] Although the properties of polyphenol oxidases from broad beans were investigated severalfold[18] and their cDNAs already cloned,[19] it could not be shown that these enzymes catalyze the conversion of tyrosine to dopa.[16] The currently demonstrated occurrence of *N*-[(−)-jasmonoyl]-(*S*)-dopa (**3**) and *N*-[(−)-jasmonoyl]-dopamine (**5**) in broad bean flowers led to the question whether their formation by hydroxylation may take place directly at the corresponding JA conjugates. Therefore, in vitro experiments to hydroxylate *N*-[(±)-jasmonoyl]-(*S*)-tyrosine [(±)-**2**] and *N*-[(±)-jasmonoyl]tyramine [(±)-**4**] by enzyme extracts from broad bean flowers and other tyrosinase preparations were performed. The isolation and identification of new JA conjugates, their chemical and enzymic synthesis, and their biological activities are reported here.

Results and Discussion

Isolation and Characterization of JA and CA Conjugates. From the MeOH extracts of broad bean flowers the compounds **2** and **3** were isolated by a combination of ion exchange chromatography on DEAE-Sephadex A25 (IEC) and preparative reversed-phase (RP) HPLC. The purified fractions were analyzed by LC-ESIMS in the positive ion mode as described earlier.[20,21] The detection of the known compound **2** in the acidic fraction of the IEC was performed by ESI-CIDMS, showing the $[M + H]^+$ ion at *m/z* 374 and typical fragments at *m/z* 182, 165, 151, and 136, being in accordance with data described earlier.[4] This fraction also yielded the new compound *N*-[(−)-jasmonoyl]-(*S*)-dopa (**3**), containing two hydroxyl groups on the aromatic ring. Compound **3** is characterized by an $[M + H]^+$ ion at *m/z* 390 and key fragments at *m/z* 344 ($M + H - HCO_2H$), 198 [**b** + 2H], 181 [**c** + H], and 152 [**b** + 2H − HCO_2H] (Figure 1). The base peak at *m/z* 151 [**a**] comprises the jasmonoyl moiety. The LC-MS data of permethylated **3** showed the $[M + H]^+$ ion at *m/z* 432. The characteristic fragments at *m/z* 240 [**b** + 2H], 223 [**c** + H], and 180 [**b** + 2H − HCO_2CH_3] were also shifted toward higher masses. The fragmentation data of **3** were found to be identical with those from *N*-[(−)-jasmonoyl]-(*S*)-dopa obtained by partial

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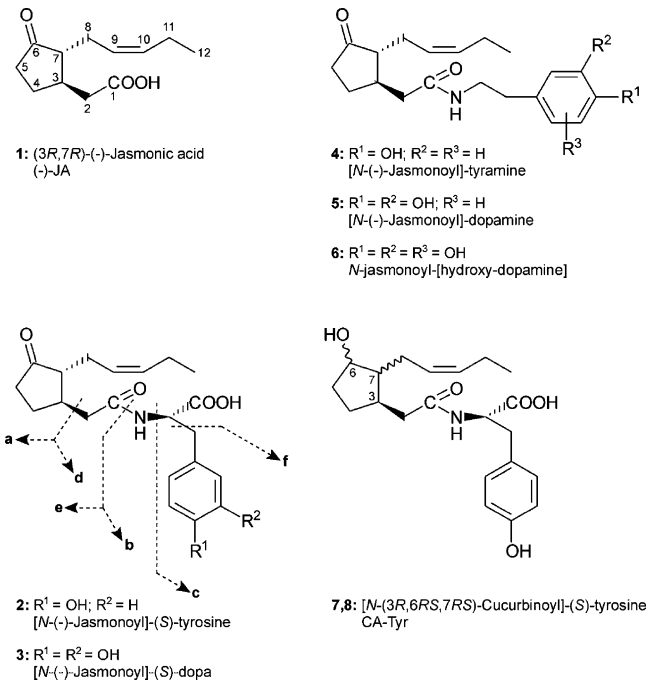

Figure 1. Structure of amide conjugates of jasmonic acid (JA) and of cucurbic acid (CA) type as well as their mass spectrometric fragmentation pattern.

synthesis. The configuration of **2** and **3** could be determined by separation on a RP-18 column under isocratic elution conditions described for synthetic JA−amino acid conjugates.[22]

Detailed analysis of the LC-MS data of the same methylated fraction revealed additionally trace signals of two tyrosine conjugates occurring at 17.4 min (**7**) as well as 18.3 min (**8**). Both compounds yielded the $[M + H]^+$ ions at *m/z* 404, two mass units higher than that of **2**. Such shifts were previously observed for the tryptophan conjugate of cucurbic acid isomer[5] and 7-isocucurbinoylisoleucine[7] containing in the C-6 position a hydroxyl group instead of the carbonyl function (see Figure 1). Fragmentation of *m/z* 404 leads to the ion at *m/z* 326 ($[M + H - HCO_2CH_3 - H_2O]^+$) compared to *m/z* 324 for **2**, indicating cucurbic acid isomers. The fragments characterizing the tyrosine moiety of the conjugates **7** and **8** were identical with those found for **2**. The configuration at C-6 and C-7 of **7** and **8** could not be determined due to lack of sufficient material.

From the neutral fraction of IEC and further purification strong evidence for the occurrence of *N*-[(−)-jasmonoyl]-dopamine (**5**) was obtained. The LC-ESIMS data showed an $[M + H]^+$ ion at *m/z* 346, 16 mass units higher as compared to the JA−tyramine conjugate detected in pollen of petunia[9] and indicated the presence of an additional hydroxyl group. The fragmentation of **5** revealed this change of the main fragment ions (Figure 1). While the elimination of the acetyl side chain of the JA residue yields a signal at *m/z* 196 [**d** + 2H] instead of *m/z* 180 as described for JA−tyramine,[9] the cleavage of the amide bond leads to the ion [**b** + 2H] at *m/z* 154 (138 for JA−tyramine). The ion of type [**c** + H] characterizing the amine moiety appears at *m/z* 137 (121 for JA−tyramine). The typical fragments comprising the JA part of the conjugate are also observed at *m/z* 193 [**e**], 151 [**a**], and 133 [**a** − H_2O]. The fragmentation pattern of **5** was identical with that of a synthetic reference compound. Methylated **5** showed similar fragments in CID, confirming the presence of two O-methyl groups. To get information concerning the configuration of the jasmonoyl moiety of **5**, the isolated conjugate was

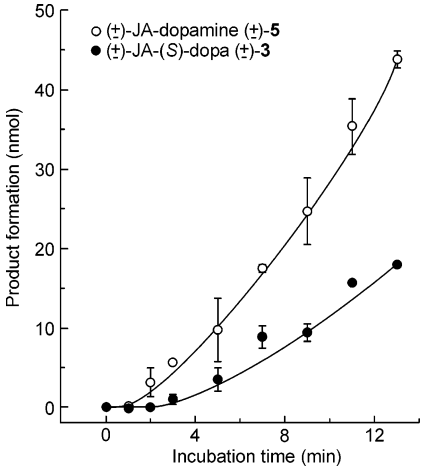

Figure 2. Time course of the transformation of (±)-**2** and (±)-**4** by mushroom tyrosinase (mean ± absolute deviation of the mean).

subjected to chiral HPLC (Chiralpak AS, isocratic elution).[23] The conjugate **5** eluted at a retention time identical with synthesized authentic *N*-[(−)-jasmonoyl]dopamine.

Enzymic Hydroxylations of (±)-2 and (±)-4. As the 3-hydroxylated derivatives of **2** and **4** were found in broad bean flowers, the question arose whether (±)-**2** and (±)-**4** are substrates for the hydroxylating activity of tyrosinases. The results summarized in Table 1 and Figure 2 indicate that both (±)-**2** and (±)-**4** are transformed to (±)-**3** and (±)-**5**, respectively, by commercial mushroom tyrosinase, but compound (±)-**4** with a 2.4-fold higher rate as compared to (±)-**2**. The analysis of the composition of the remaining substrates of the assays and the products formed on a chiral column revealed that there was no preference for the (+)- or (−)-JA conjugates by the enzyme. This may be due to the distance of the JA part from the site of transformation. The hydroxylation results may also be interpreted as a hint for the possible biosynthetic formation of **3** and **5** in *V. faba* in the same way. Therefore, enzyme extracts from broad bean flowers were tested with the same substrates (±)-**2** and (±)-**4**. The results showed that both (*S*)-tyrosine and (±)-**2** are hydroxylated to (*S*)-dopa and (±)-**3**, respectively, with similar low rates, but (±)-**4** with an 11.2-fold higher rate as compared to (±)-**2** (Table 1). In addition, a tyrosinase-containing extract from hairy roots of red beet[24] hydroxylated besides (*S*)-tyrosine also (±)-**2** and (±)-**4** in a relation similar to mushroom tyrosinase (Table 1). Although the involvement of plant tyrosinases/polyphenol oxidases in the biosynthesis of different groups of secondary compounds (betalains,[24,25] aurones,[26−28] lignans[29]) in regiospecific or enantiospecific manner is known, fungal tyrosinases (*Agaricus bisporus*,[30] *Neurospora crassa*[26]) do not generally show a strict substrate specificity. Both in the mushroom tyrosinase-catalyzed transformation of (±)-**4** and in long-term incubations (2 h) with the protein extract from broad bean flowers, the formation of an additional product more polar than (±)-**5** was observed. This compound (**6**) exhibited UV properties similar to those of (±)-**5**, but the ESIMS yielded an $[M + H]^+$ ion at *m/z* 362, 16 mass units higher compared to (±)-**5** (*m/z* 346), indicating the presence of an additional hydroxyl group possibly attached to the aromatic ring. The other main fragments are shifted similarly (Table 2). Treatment of **6** with diazomethane led to the formation of a derivative generating an $[M + H]^+$ ion at *m/z* 404, confirming the methylation of three phenolic hydroxyl groups. A hydroxyl group located at the aliphatic side chain of dopamine was not methylated under these conditions. A similar com-

Table 1. Hydroxylation Activity of Tyrosinase and Plant Protein Extracts toward (*S*)-Tyrosine, (±)-JA−(*S*)-Tyrosine [(±)-**2**], and (±)-JA−Tyramine [(±)-**4**]

enzyme source	specific activity (pkat/mg protein)[a]			
	(*S*)-Tyr	(±)-(**2**)	(±)-(**4**)	ratio (±)-**4**/(±)-**2**
tyrosinase (mushroom, Sigma)	15 637 ± 156[b]	2318 ± 36	5631 ± 141	2.4
Vicia faba flowers	83 ± 6	77 ± 29	868 ± 68	11.2
B. vulgaris hairy roots	1390 ± 30	852 ± 110	1610 ± 25	1.9

[a] Mean value ± absolute deviation of the mean. [b] With tyramine as substrate: 6637 ± 561 pkat/mg protein.

Table 2. CID MS Data Derived from the Protonated Molecular Ion of **4**, **5**, and **6** [*m/z* (relative intensity)]

ion	(±)-**4** synthetic	(±)-**5** enzymic	(±)-**6** enzymic
$[M + H]^+$	330 (5)	346 (3)	362 (4)
$[M + H - H_2O]^+$	312 (4)	328 (5)	344 (3)
(**d** + 2H)	180 (7)	196 (9)	212 (3)
e		193 (5)	193 (14)
(**e** − H_2O)			175 (5)
$(\mathbf{b} + 2H)^+$	138 (11)	154 (30)	170 (36)
(**c** + H)	121 (100)	137 (85)	153 (100)
a	151 (62)	151 (100)	151 (73)
(**a** − H_2O)	133 (34)	133 (35)	133 (22)

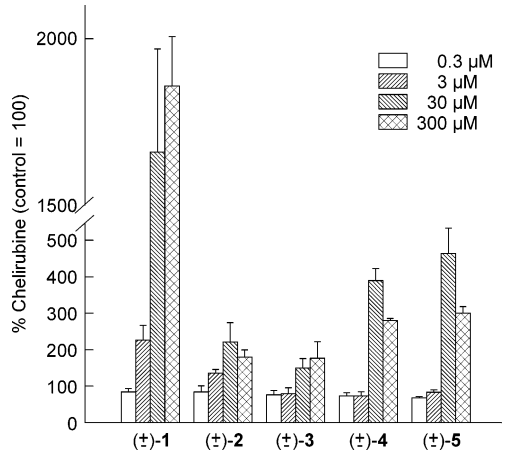

Figure 3. Effect of JA conjugates and JA on elicitation of chelirubine formation in cell cultures of *E. californica* (80% ethanol control: 100%) (mean ± absolute deviation of the mean).

pound, 6-hydroxydopa, formed by water addition to the 6-position of protein-bound dopa-quinone, was found to be the precursor of trihydroxyphenylalanine quinone (topaquinone), which was identified as an essential cofactor of copper amine oxidase.[31] Therefore, the structure of the minor product of enzymic hydroxylation of (±)-**4** is proposed to be (±)-JA-(6-hydroxydopamine), formed in a similar way.

Concerning the enzymic hydroxylation of (±)-**2** and (±)-**4** using a crude protein extract from flowers, it is not certain whether both transformations were catalyzed necessarily by the same enzyme, because in callus cultures of *Portulaca grandiflora* different enzymes for the hydroxylation of tyrosine and tyramine have been characterized.[24]

The characterization of new JA amide conjugates raised the question concerning their biological activities. Thus, (±)-**2**, (±)-**3**, (±)-**4**, and (±)-**5** were tested in comparison to JA [(±)-**1**] with cell suspensions of *Eschscholzia californica* Cham. for alkaloid-eliciting activity.[32] The formerly used photometric determination of the alkaloid formation at 490 nm was replaced by an HPLC analysis of the major compound chelirubine, whose peak areas were compared with controls without elicitors. The results (Figure 3) show that the eliciting activities of (±)-**2**, (±)-**3**, (±)-**4**, and (±)-**5** were strongly reduced as compared to (±)-**1**, but the amine conjugates (±)-**4** and (±)-**5** exhibited slightly higher activities at 30 and 300 µM than the corresponding JA amino acid conjugates (±)-**2** and (±)-**3**. Whether these compounds are biologically active per se or due to hydrolysis in the bioassay system awaits further studies.

The low biological activities of **2**, **3**, **4**, and **5** do not exclude a signaling function in the flower development of broad beans. The induction of specific genes in barley leaves upon treatment with different JA−isoleucine isomers indicated that the native *N*-[(−)-jasmonoyl]-(*S*)-isoleucine was most active.[6b] The biosynthesis of amino acid conjugates proceeds in general by the linkage of JA[33] or the auxin indole-3-acetic acid[34] with different amino acids as recently shown. The formation of **3** and **5** by in vitro hydroxylation of **2** and **4** with the broad bean flower enzyme could be a hint to an alternative biosynthesis of these JA amide conjugates.

Experimental Section

General Experimental Procedures. The LC/ESIMS and ESIMS/MS measurements were performed on a Finnigan MAT TSQ 7000 instrument as described previously.[20] The LC was carried out as follows: column LiChrospher 100 RP-18 (100 × 2 mm, 5 µm); mobile phase MeCN/H_2O (containing 0.2% HOAc); flow rate 0.2 mL min^{-1}; injection volume 2 µL. The collision-induced dissociation (CID) mass spectra of the $[M + H]^+$ ions were performed during the HPLC run at a collision energy of 25 eV (collision gas argon, collision pressure 1.8 mTorr). All mass spectra were averaged and background subtracted. HPLC was performed with a system from Waters (Milford, MA), including the separation module 2690. The liquid chromatograph was equipped with a 5 µm Nucleosil C_{18} column (250 × 4 mm i.d.; Macherey-Nagel, Düren, Germany), and the following solvents and systems were used. Solvents: A, 0.2% aqueous HOAc; B, MeOH; *System 1*: isocratic elution at 55% A/45% B (for the separation of stereoisomers); *System 2*: isocratic elution at 45% A/55% B (for the separation of the products of the enzyme assays); *System 3*: isocratic elution at 55% A/45% B for 25 min, then within 10 min to 10% A/90% B (for the separation of JA−amino acids and their hydroxylated products). The flow rate was 1 mL min^{-1}. The compounds were detected at 210 and 280 nm as well as by Maxplot detection between 200 and 400 nm (photodiode array detection). Additionally, a fluorescence detector (excitation 280 nm; emission 314 nm) was used.

Racemic jasmonic acid [(3*R*,7*R*/3*S*,7*S*)-(±)-JA, (±)-**1**] was obtained by saponification of its methyl ester purchased from Firmenich (Geneva, Switzerland). All other chemicals were commercially available (Merck, Sigma).

Plant Material. Broad bean plants (*V. faba* L. cv. Scirocco) were grown from seeds in a greenhouse and cultivated in soil under natural daylight conditions until flowering. A voucher seed specimen was deposited (RK-100) in the seed center of the Institute.

A red beet hairy root culture [*Beta vulgaris* L. subsp. *vulgaris* (Garden Beet Group) "Egyptian Flatround"] was grown in the dark at 25 °C and maintained as described recently.[24]

Synthesis of Reference Compounds. The authentic standards (±)-**2**, (±)-**3**, (±)-**4**, and (±)-**5** were synthesized by reaction of (±)-**1** with (*S*)-tyrosine, (*S*)-dopa, tyramine, and dopamine, respectively, in 75−85% yields according to our procedure.[22,35] The resulting diastereomeric pairs of (±)-**2** as

well as of (±)-**3** were resolved by HPLC on a RP18-column (250 × 4 mm, 5 μm, flow rate of 1 mL min^{-1}, UV detection at 210 nm) using a mobile phase consisting of 35% (v/v) MeOH/H_2O (containing 0.2% HOAc). Retention times (t_R) were as follows: (+)-**3**, 31.4 min; (−)-**3**, 33.9 min, (+)-**2**, 54.3 min, (−)-**2**, 61.1 min. The resolution of the respective enantiomeric pairs of (±)-**4** as well as (±)-**5** performed on a Chiralpak AS (Daicel, J. T. Baker, Gross Gerau, Germany) column (250 × 4.6 mm, 5 μm, flow rate of 1 mL min^{-1}, UV detection at 210 nm) with a 4:1 (v/v) mixture of *n*-hexane and 2-propanol yielded the enantiomers at the indicated t_R: (−)-**5**, 35.4 min; (+)-**5**, 41.8 min; (−)-**4**, 38.1 min; (+)-**4**, 47.4 min.

Isolation and Purification of JA Conjugates from Broad Bean Flowers. Fully developed flowers (17 g fresh weight) of *V. faba* were harvested, frozen in liquid nitrogen, and stored at −80 °C prior to homogenization and extraction with 15 mL of 80% (v/v) aqueous MeOH. The extract was separated on a DEAE-Sephadex A-25 column (25 mL gel, acetate form) using a discontinuous gradient of HOAc in MeOH (40 mL of MeOH, 40 mL of 0.04 M HOAc in MeOH, 40 mL of 1.5 M HOAc in MeOH).

The first two fractions (neutral fraction) were combined, evaporated, and subsequently purified by RP18-HPLC using a 1:1 (v/v) mixture of MeOH and 0.2% (v/v) aqueous HOAc as mobile phase. The fractions corresponding to the authentic substances (±)-**5** (11−14 min) and (±)-**4** (17−19 min) were pooled, concentrated in vacuo, and stored at −20 °C prior to the LC/MS analysis. The acidic fraction from IEC was concentrated in vacuo and subjected to RP18-HPLC with the mobile phase consisting of a 1:1 (v/v) mixture of MeOH and 0.2% (v/v) aqueous HOAc. The fractions corresponding to authentic reference substances (±)-**3** (7−9 min) and (±)-**2** (10−12 min) were pooled, concentrated in vacuo, and stored at −20 °C prior to the LC/MS analysis. For further purification, the samples were redissolved in MeOH, treated with diazomethane, and then purified on cartridges (500 mg LiChrolut RP-18, Merck, Darmstadt) preequilibrated with 10% (v/v) MeOH in 0.2% (v/v) aqueous HOAc. Nonmethylated products were removed by washing with 2 mL of the mobile phase, and the methylated products were eluted with 6 mL of 60% (v/v) MeOH in aqueous HOAc. The eluates were concentrated in vacuo and stored at −20 °C prior to the LC/MS analysis, respectively.

Preparation of Enzyme Extracts. Broad bean flowers and hairy root material (*B. vulgaris*) were homogenized and processed according to Steiner et al.[24] Protein content was determined by the Bradford dye-binding method[36] using human serum albumin as a standard.

Hydroxylation of (±)-JA Conjugates by Mushroom Tyrosinase. To 50 nmol of JA conjugates [(±)-**2**, (±)-**4**] was added 20 μL of tyrosinase [(mushroom, Sigma), 1 mg mL^{-1} potassium phosphate buffer (25 mM, pH 5.8, containing 100 mM Na-ascorbate/ascorbic acid)], and the mixture was incubated in a thermomixer at 30 °C for 15 min. Then 20 μL of MeOH was added, the mixture was centrifuged (15.000*g*, 5 min, 4 °C), and 30 μL of the supernatants was analyzed by HPLC (*system 2*).

Controls without tyrosinase were included. All assays were performed in duplicate.

For measuring the kinetics of the hydroxylation of (±)-**2** and (±)-**4** by tyrosinase, 200 nmol of the substrates was dissolved in 200 μL of Na-ascorbate/ascorbic acid (100 mM, pH 5.8) and the reaction started by the addition of 200 μL of tyrosinase (50 μg mL^{-1}). After 1, 2, 3, 5, 7, 9, 11, and 13 min, two 10 μL aliquots were removed from the incubation mixture and added to 30 μL of MeOH. After centrifugation 25 μL each was used for HPLC analysis (*system 2*). The percentages of the formed products calculated from the peak areas were plotted against the incubation time. The hydroxylation of (*S*)-tyrosine and tyramine by mushroom tyrosinase was done for comparison as described recently,[24] and the products dopa and dopamine were analyzed by HPLC (*system 2*, fluorescence detection).

Hydroxylation of (±)-JA Conjugates by Protein Extracts from Broad Bean Flowers and Red Beet Hairy Root Cultures. Substrates [(±)-**2**, (±)-**4**, 30 nmol] were dissolved in 20 μL of Na-ascorbate/ascorbic acid (200 mM, pH 5.7). After preincubation (30 °C, 5 min), the reaction was started by the addition of 20 μL of protein extract [(20 mM KPi, pH 5.7, 5 mM ascorbate, 20 μM $CuCl_2$) (final substrate concentration 0.75 mM)]. The reactions were stopped after 10 min by the addition of 40 μL of MeOH, the mixture was centrifuged, and 60 μL of the supernatants was analyzed by HPLC (*system 2*). For comparison (*S*)-Tyr was hydroxylated under the same conditions to yield (*S*)-dopa.

Elicitor Activity Assay. The bioassay using a cell culture of *Eschscholzia californica* Cham. was performed in triplicate as described.[32] For chelirubine analysis, the acidic extracts were analyzed by HPLC [column LiChrospher 60 RP-select B (250 × 4 mm, 5 μm); solvent A 98% H_2O, 2% MeCN; solvent B 98% MeCN, 2% H_2O (both containing 0.01% H_3PO_4); UV detection at 490 nm; flow rate of 1 mL min^{-1}; gradient from 100/0 to 40/60 in 25 min followed by 5 min isocratic elution]. Authentic chelirubine eluted at t_R 28.5 min. The chelirubine content of controls was set to 100%.

***N*-[(3*R*,7*R*)-(−)-Jasmonoyl]-(*S*)-tyrosine (2):** t_R 18.4 min, ESI-CIDMS *m/z* (%) 374 $[M + H]^+$ (39), 328 $[M + H - HCO_2H]^+$ (61), 310 $[M + H - HCO_2H - H_2O]^+$ (11), 251 (3), 222 [**d** + H] (14), 193 [**e**] (9), 182 [**b** + 2H] (48), 178 [**d** + H − HCO_2H] (13), 165 [**c** + H] (52), 151 [**a**] (100), 136 [**b** + 2H − HCO_2H] (72), 133 [**a** − H_2O] (15), 109 (4), 107 [**f**] (9), 93 (8).

***N*-(3*R*,7*R*)-(−)-Jasmonoyl]-(*S*)-tyrosine (2) (methylated):** t_R 21.1 min, ESI-CIDMS *m/z* (%) 402 $[M + H]^+$ (3), 342 $[M + H - HCO_2Me]^+$ (100), 324 $[M + H - HCO_2Me - H_2O]^+$ (11), 210 [**b** + 2H] (24), 193 [**c** + H and/or (**e**)] (40), 192 [**d** + H − HCO_2H] (24), 175 (5), 151 [**a**] (61), 150 [**b** + 2H − HCO_2-Me] (82), 133 [**a** − H_2O] (5) 121 [**f**] (14).

***N*-(3*R*,7*R*)-(−)-Jasmonoyl]-(*S*)-dopa (3):** t_R 13.2 min, ESI-CIDMS *m/z* (%) 390 $[M + H]^+$ (3), 344 $[M + H - HCO_2H]^+$ (29), 326 $[M + H - HCO_2H - H_2O]^+$ (4), 210 (5), 198 [**b** + 2H] (67), 193 [**e**] (11), 181 [**c** + H] (23), 152 [**b** + 2H − HCO_2H] (74), 151 [**a**] (100), 147 (10), 133 [**a** − H_2O] (17), 93 (6).

***N*-(3*R*,7*R*)-(−)-Jasmonoyl]-(*S*)-dopa (3) (methylated):** t_R 17.2 min, ESI-CIDMS *m/z* (%) 432 $[M + H]^+$ (3) 372 $[M + H - HCO_2Me]^+$ (100), 354 $[M + H - HCO_2Me - H_2O]^+$ (4), 240 [**b** + 2H] (56), 223 [**c** + H] (99), 193 [**e**] (26), 151 [**a**] (69), 180 [**b** + 2H − HCO_2Me] (39), 133 [**a** − H_2O] (6).

***N*-(3*R*,7*R*)-(−)-Jasmonoyl]dopamine (5):** t_R 14.4 min, ESI-CIDMS *m/z* (%) 346 $[M + H]^+$ (4), 328 $[M + H - H_2O]^+$ (5), 196 [**d** + 2H] (9), 180 (8), 167 (16), 154 [**b** + 2H] (16), 151 [**a**] (46), 137 [**c** + H] (100), 133 [**a** − H_2O] (40), 107 (6), 93 (17).

***N*-(3*R*,7*R*)-(−)-Jasmonoyl]dopamine (5) (methylated):** t_R 17.4 min, ESI-CIDMS *m/z* (%) 374 $[M + H]^+$ (3), 356 $[M + H - H_2O]^+$ (2), 224 [**d** + 2H] (2), 193 [**e**] (39), 182 [**b** + 2H] (8), 165 [**c** + H] (100), 151 [**a**] (64), 133 [**a** − H_2O] (15).

Compound 7 (methylated): t_R 17.4 min, ESI-CIDMS *m/z* (%) 404 $[M + H]^+$ (3), 386 $[M + H - H_2O]^+$ (4), 326 $[M + H - H_2O - HCO_2Me]^+$ (17), 267 (4), 210 [**b** + 2H] (79) 193 [**c** + H] (100), 177 (15), 150 [**b** + 2H − HCO_2Me] (30), 135 (8), 121 [**f**] (12).

Compound 8 (methylated): t_R 18.3 min, ESI-CIDMS *m/z* (%) 404 $[M + H]^+$ (3), 386 $[M + H - H_2O]^+$ (5), 326 $[M + H - H_2O - HCO_2Me]^+$ (13), 267 (4), 210 [**b** + 2H] (65), 193 [**c** + H] (100), 177 (17), 150 [**b** + 2H − HCO_2Me] (39), 135 (10), 121 [**f**] (21).

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