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Amino Acid Conjugates as Metabolites of the Plant Growth Regulator Dihydrojasmonic Acid in Barley (*Hordeum vulgare*)

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Abstract. The biotransformation of [2-^{14}C](±)9,10-dihydrojasmonic acid (DJA) was studied in excised shoots of 6-day-old barley seedlings after 72 h. From the ethyl acetate extract, some minor metabolites were isolated and purified by DEAE-Sephadex A-25 chromatography, thin-layer chromatography (TLC), C_{18}-cartridges, and high-performance liquid chromatography (HPLC). The structural identification of these metabolites was performed by gas chromatography–mass spectrometry (GC-MS), circular dichroism (CD), and amino acid analysis, and the following amino acid conjugates were found: *N*-[(−)9,10-dihydrojasmonoyl]valine, *N*-[(−)9,10-dihydrojasmonoyl]isoleucine, *N*-[9,10-dihydrojasmonoyl]leucine, *N*-[11-hydroxy-9,10-dihydrojasmonoyl]valine, *N*-[11-hydroxy-9,10-dihydrojasmonoyl]isoleucine, *N*-[12-hydroxy-9,10-dihydrojasmonoyl]isoleucine; and the cucurbic acid-related compounds *N*-{[3-hydroxy-2(4-hydroxypentyl)-cyclopent-1-yl]-acetyl}isoleucine and *N*-{[3-hydroxy-2(5-hydroxypentyl)-cyclopent-1-yl]-acetyl}isoleucine. The results suggest conjugation with isoleucine and valine, as well as preferential hydroxylation at position C-11 or hydrogenation at position C-6, as being important steps in the metabolism of (±)DJA in barley shoots.

(−)Jasmonic acid [(−)JA] and its methyl ester (Fig. 1) have been found to be widely distributed in plants (Meyer et al. 1984) and are considered to be representatives of a new type of endogenously occurring plant growth regulator with hormone-like properties (see for example, Gross 1990, Sembdner and Gross 1986, Sembdner and Klose 1985). A few studies concerning the biosynthesis of JA have been published (Vick and Zimmerman 1983, 1984). However, the knowledge of its metabolism is still very limited (Jacobus 1986, Kehlen 1987, Schröder 1982). Reports on plant growth-regulating activities of 9,10-dihydrojasmonic acid (DJA, Fig. 1) (Ravid et al. 1975, Ueda et al. 1981, Yamane et al. 1980) and its natural occurrence in fruits of *Vicia faba* (Miersch et al. 1989) caused us to study the metabolism of radiolabeled (±)DJA in barley seedlings. In an earlier report (Meyer et al. 1989), we described the identification of the major metabolites as (−)11-hydroxy-9,10-DJA and its O-β-D-glucopyranoside, besides some other kinds of metabolites. A lesser amount of (−)12-hydroxy-9,10-DJA was also found. This paper reports on the structural elucidation of eight minor metabolites (amino acid conjugates) of (±)DJA.

Materials and Methods

Authentic Substances

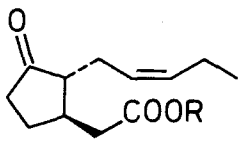
(±)DJA was obtained by alkaline hydrolysis of (±)DJA methyl ester (Firmenich, Geneva, Switzerland). [2-^{14}C](±)DJA (2.1 mCi/mM) was synthesized by the method of Unverricht and Gross (1986).

Plant Material and Feeding Experiments

Feeding experiments were carried out according to Meyer et al. (1989) using shoots of 6-day-old barley seedlings (*Hordeum vulgare L.* cv. "Certina").

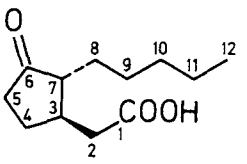
Detection of Radioactivity

Radioactivity was measured by liquid scintillation counting in a Tricarb Modell 2660 (Packard, Dowers Grove, IL, USA). Thin-layer chromatography (TLC) plates were monitored with a radioscanner (Berthold, Wildbad, FRG).

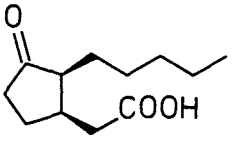


R = H (-)-Jasmonic acid

R = CH_3 (-)-Methyl jasmonate



(-)-9,10-Dihydrojasmonic acid



(+)-7-iso-9,10-Dihydrojasmonic acid

Fig. 1. Chemical structures of JA and related compounds.

Isolation of DJA Metabolites

The barley shoots were homogenized and extracted in the same way as described by Meyer et al. (1989).

The combined ethyl acetate extract was chromatographed on a DEAE-Sephadex A-25 column (50 × 2.0 cm) using a discontinuous gradient of acetic acid in 80% aqueous methanol according to Gräbner et al. (1976). Fractions eluted with 0.75 N acetic acid in 80% methanol were subjected to TLC in system 1 (three times), followed by a development in system 2 (one time), yielding two main radioactive zones A and B at R_f = 0.65 and 0.26, respectively. Both zones were eluted with methanol.

Zone A was further purified on a Sep-Pak C_{18}-cartridge with an increasing gradient of methanol in 0.2% acetic acid giving the fraction of A-type metabolites (50–60% methanol). It was finally separated by high-performance liquid chromatography (HPLC) in system 1 into three radioactive compounds: A1 (R_t = 14.8 min), A2 (R_t = 22.9 min), and A3 (R_t = 26.5 min). A3 was applied to TLC in system 3 (R_f = 0.60) and compared with *N*-[jasmonoyl]leucine (R_f = 0.60) used as the standard. Compounds A1 and A2 after HPLC and A3 after TLC were methylated and structural identification accomplished by gas chromatography–mass spectrometry (GC-MS). Additionally, these compounds were investigated regarding their Cotton effects by circular dichroism (CD) measurements.

The fraction of B-type metabolites was rechromatographed on TLC (system 4) resulting in three radioactive zones: B1 (R_f = 0.68, B2 (R_f = 0.61), and B3 (R_f = 0.51).

B1 and B2 were eluted separately from the plate with methanol and finally purified using Sep-Pak C_{18}-cartridges. An increasing gradient of methanol in 0.2% acetic acid eluted both B1 and B2 in the 30–40% methanol-containing fraction. Amino acid analysis was performed on aliquots. The major part was methylated with diazomethane and trimethylsilylated for capillary GC-MS analysis, resulting in the three products: B1-Me-TMS, B2a-Me-TMS, and B2b-Me-TMS.

Metabolite B3 after TLC in system 4 was methylated and supplied to a further TLC (R_f = 0.76). One part of the sample was directly investigated by capillary GC-MS, giving the peaks B3a-Me and B3b-Me. The remaining part was additionally trimethylsilylated and studied by GC-MS giving the corresponding spectra of the metabolites, B3a-Me-TMS and B3b-Me-TMS.

TLC

For TLC on activated silica gel GF_{254} (Merck, Darmstadt, FRG), the following solvent systems were used: (1) benzene–ether–acetic acid (40:9:1 by volume), three times; (2) chloroform–ethyl acetate–acetic acid (5:4:1 by volume), one time; (3) chloroform–ethyl acetate–acetic acid (14:6:1 by volume), two times; (4) chloroform–methanol–acetic acid–water (80:20:3:2 by volume), one time.

Detection was performed by radioscanning or spraying with anisaldehyde reagent and heating for 5–10 min at 120°C (Stahl and Glatz 1982). Zones of interest were eluted with methanol from the plates.

Purification on Sep-Pak C_{18}-Cartridges

The amino acid conjugate fractions A, B1, and B2 after TLC were further purified on silica Sep-Pak C_{18} reverse-phase prepacked cartridges (Waters Associates GMBH, Königstein, FRG). The cartridge was rinsed first with 7 ml methanol and 7 ml McIlvaine buffer, pH 3.0. Then the conjugate fraction was dissolved in 100 µl methanol and injected onto the cartridge. The latter was washed with 2 ml buffer, pH 3.0, and 7.5 ml 0.2% acetic acid, followed by 7.5 ml of an increasing gradient of methanol in 0.2% acetic acid (10, 20, . . . 100% methanol).

Fraction A was eluted with 50–60% methanol, metabolites B1 and B2 with 30–40% methanol in 0.2% acetic acid from the cartridge as determined by measuring the radioactivity. The regeneration of the cartridge was performed by washing with 5 ml ethyl acetate, 7 ml methanol, and 7 ml buffer, pH 3.0.

HPLC

For HPLC of fraction A an HP 1090 Chromatograph (Hewlett Packard, Vienna, Austria) was used fitted with a Hypersil RP 8 (5 µm) column (4.6 × 200 mm). For solvent system 1, 40% methanol containing 0.2% acetic acid was used at a flow rate of 0.6 ml min^{-1}; radiodetection and UV detection at 228 nm were performed.

Identification of Metabolites with Combined GC-MS

Methylated amino acid conjugates of type A were identified by GC-MS (Varian MAT, Bremen, FRG) using a steel column (1.50 m × 2 mm) with 3% UV-225 on Gaschrom Q (100–120 µm), He at 15 ml min^{-1}, column temperature at 230°C, and EID (A1-Me, R_t = 5.9 min; A2-Me, R_t = 6.8 min; A3-Me, R_t = 6.8 min).

Prior to GC-MS the methylated metabolites B1, B2, and B3 were trimethylsilylated with Sil-Prep for 60 min. Analyses were

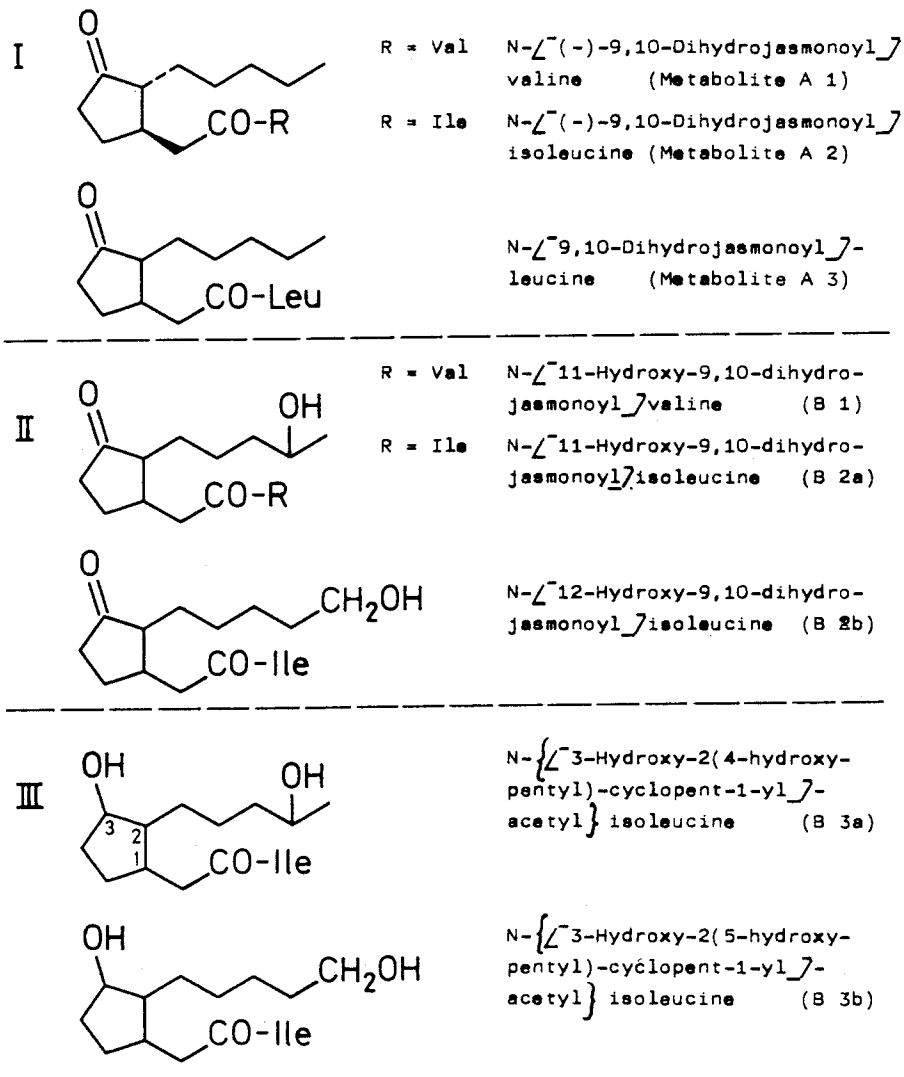


Fig. 2. Structure of amino acid conjugates as metabolites from (±)9,10-DJA in barley seedlings.

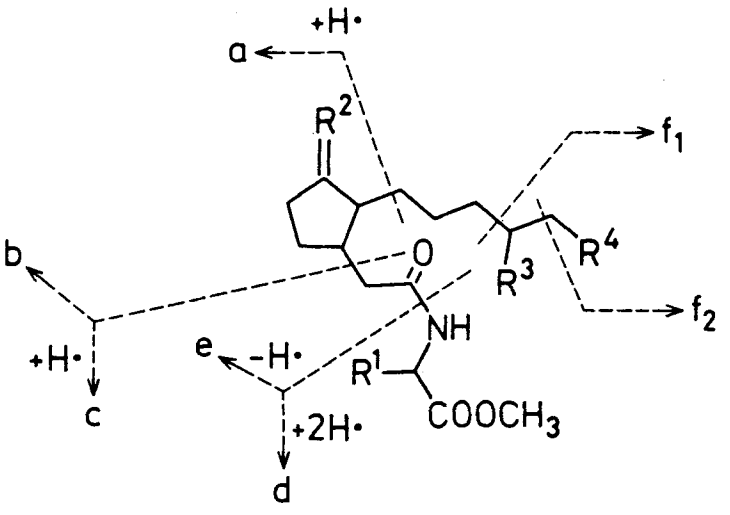
performed using capillary GC-MS (B1-Me-TMS, R_t = 16.3 min; B2a-Me-TMS, R_t = 17.5 min; B2b-Me-TMS, R_t = 18.6 min; B3a-Me, R_t = 17.1 min; B3b-Me, R_t = 18.0 min). For these studies, a Hewlett Packard quadrupole mass spectrometer (Model 5970 B) equipped with an HP 5890 gas chromatograph was used. GC was performed on a 25 m × 0.2 mm ID cross-linked methylsilicone-fused silica column, with a film thickness of 0.11 μm, helium as the carrier gas at 2.5 ml min^{-1}, and splitness injection. Temperatures were as follows: injection, 275°C; direct inlet interface, 230°C; and ion source, 250°C. The temperature program was as follows: from 60°C (1 min) to 180°C (30°C min^{-1}); from 180°C (1 min) to 200°C (10°C min^{-1}); from 200°C (1 min) to 270°C (5°C min^{-1}). The electron impact energy was 70 eV. The GC-MS system was combined with an HP 9000/300-9133 computer set. The background-corrected average mass spectra of the methylated or trimethylsilylated compounds were used for the discussion of fragmentation.

Amino Acid Analyses

After purification on DEAE-Sephadex A-25 and by TLC (sys-

Table 1. Key ions of the mass spectra of the methylated N-(dihydrojasmonoyl) amino acid conjugates, type I.

ion	A1-Me m/z (relat. Int. %)	A2-Me m/z (relat. Int. %)	A3-Me m/z (relat. Int. %)
$M^{+\cdot}$	325 (22)	339 (3)	339 (3)
$[M\text{-}\dot{O}CH_3]^+$	294 (5)	—	—
$[M\text{-}57]^+$	268 (10)	282 (3)	282 (3)
$[M\text{-}\dot{C}OOCH_3]^+$	266 (12)	280 (3)	280 (3)
a, $[M\text{-}70]^{+\cdot}$	255 (15)	269 (2)	269 (3)
e	194 (12)	—	—
c	173 (93)	187 (28)	187 (20)
m/z 156	—	(8)	(8)
b	153 (25)	153 (11)	153 (8)
d	132 (82)	146 (28)	146 (28)
$(c\text{-}C_4H_8)$	—	131 (38)	131 (88)
$(c\text{-}\dot{C}OOCH_3)$	114 (85)	128 (73)	128 (19)
m/z 99	(49)	(13)	(21)
m/z 96	(30)	(22)	(11)
$(d\text{-}HCOOCH_3)$	72 (100)	86 (100)	86 (100)



compound	R^1	R^2	R^3	R^4
A1-Me	$CH(CH_3)_2$	O	H	H
A2-Me	$CH(CH_3)C_2H_5$	O	H	H
A3-Me	$CH_2CH(CH_3)_2$	O	H	H
B1-Me-TMS	$CH(CH_3)_2$	O	OTMS	H
B2a-Me-TMS	$CH(CH_3)C_2H_5$	O	OTMS	H
B2b-Me-TMS	$CH(CH_3)C_2H_5$	O	H	OTMS
B3a-Me	$CH(CH_3)C_2H_5$	H,OH	OH	H
B3b-Me	$CH(CH_3)C_2H_5$	H,OH	H	OH
B3a-Me-TMS	$CH(CH_3)C_2H_5$	H,OTMS	OTMS	H
B3b-Me-TMS	$CH(CH_3)C_2H_5$	H,OTMS	H	OTMS

Fig. 3. Mass spectral fragmentation of the amino acid conjugates of (±)9,10-DJA and related compounds.

tems 1 and 2), aliquots of the amino acid conjugate fractions A and B were hydrolyzed in 2 ml 6N HCl in sealed tubes at 110°C for 24 h. HCl was removed by vacuum distillation and drying over NaOH in a vacuum desiccator. The residue was dissolved in buffer, pH 2.2. The quantitative determination of the amino acids was performed on the Beckman Amino Acid Analyzer 119 CL according to Spackman et al. (1958) using ion-exchange resin M 81 and sodium citrate buffers, pH 3.25 and 4.25. The amino acids valine (R_t = 48.0 min), isoleucine (R_t = 64.1 min), and leucine (R_t = 66.1 min) have been identified by cochromatography of the corresponding reference substances.

CD

CD measurements were performed on a Model 60 (Cary, USA) with CD attachment using 1-mm quartz cuvettes.

Results

After a feeding period of 72 h, the radiolabeled (±)DJA was taken up by excised barley shoots to about 90%. The radioactivity was almost completely extracted by methanol from the plant material. After evaporation of the methanol, the remaining aqueous phase was partitioned with n-hexane (hexane extract discarded) and then at pH 2.5 with ethyl acetate. Besides the major metabolites (about 90% of radioactivity) published by Meyer et al. (1989), some minor compounds (~6% of radioactivity) described herein were found to be completely extracted by ethyl acetate. Purification on a DEAE-Sephadex A-25 column gave one radioactive fraction eluted with 0.75 N acetic acid in methanol. After separation on TLC in different solvent systems, Sep-Pak C_{18}-cartridges and in one case (fraction A) by HPLC, six fractions were finally obtained (A1, A2, A3, B1, B2, and B3). By GC a further splitting of B2 to the compounds B2a and B2b and of B3 to B3a and B3b was observed, so that in summary eight metabolites could be identified (see Fig. 2). They were identified using GC-MS or capillary GC-

Table 2. Key ions of the mass spectra of methylated and trimethylsilylated N-(hydroxydihydrojasmonoyl) amino acid conjugates, Type II.

Ion	B1-Me-TMS m/z (relat. Int. %)	B2a-Me-TMS m/z (relat. Int. %)	B2b-Me-TMS m/z (relat. Int. %)
$M^{+\cdot}$	413 (3)	427 (5)	427 (12)
$[M\text{-}\dot{C}H_3]^+$	398 (32)	412 (41)	412 (42)
$[M\text{-}\dot{C}OOCH_3]^+$	—	368 (6)	368 (11)
$[M\text{-TMS}]^+$	340 (5)	340 (9)	340 (1)
$[M\text{-TMS OH}]^+$	323 (16)	337 (20)	337 (2)
a	255 (17)	269 (18)	269 (24)
b	241 (5)	241 (9)	241 (39)
$[M\text{-}\dot{C}H_3\text{-c}]$	225 (30)	225 (37)	225 (72)
197	(15)	(15)	(1)
c	173 (21)	187 (17)	187 (41)
(b-TMSOH)	151 (50)	151 (59)	151 (35)
d	132 (31)	146 (27)	146 (47)
$(c\text{-}C_4H_8)$	—	131 (11)	131 (37)
$(c\text{-}\dot{C}OOCH_3)$	114 (25)	128 (29)	128 (76)
f	117 (66)	117 (68)	103 (10)
$(d\text{-}HCOOCH_3)$	72 (89)	86 (63)	86 (100)
m/z 75	(64)	(71)	(81)
$[TMS]^+$	73 (100)	73 (100)	73 (100)

MS. It was shown by GC-MS that the metabolites belong to three types of amino acid conjugates: group 1 (A1, A2, A3, together 4% of radioactivity), group 2 (B1, B2a, B2b, together 1% of radioactivity), and group 3 (B3a, B3b, together 1% of radioactivity).

Group 1 derives itself from DJA bound to valine (A1), isoleucine (A2), and leucine (A3), respectively. The identity of the amino acid moiety in the three conjugates was proven by amino acid analyses. The ratio of conjugated valine/isoleucine/leucine was 1:1.3:0.3 as detected by HPLC and amino acid analyses. Structural elucidation of the conjugates by GC-MS was achieved preferably of the methylated compounds. The electron impact mass spectra of the three A-type amino acid conjugates (as methyl esters) are mainly characterized by their M^+ and key ions of type $[M\text{-}COOCH_3]^+$, **a, b, c, d,** and **e** (Table 1, Fig. 3). This is in accordance to the principal fragmentation of the JA amino acid conjugates (Kramell et al. 1988, Schmidt et al. 1990). The fragments **b** and **e** comprise the basic structure of DJA, whereas ions of type **c, d,** $(c\text{-}C_4H_8)$, $(c\text{-}COOCH_3)$, and $(d\text{-}HCOOCH_3)$ determine the amino acid residue. A1-Me reveals a M^+ ion at m/z 325 which corresponds to the *N*-[dihydrojasmonoyl]valine methyl ester. Ions at m/z 255 (**a**), 153 (**b**), and 194 (**e**) confirm the DJA basic structure. The most intense ions at m/z 173 (**c**), m/z 132 (**d**), m/z 114 $(c\text{-}COOCH_3)$ and m/z 72 $(d\text{-}HCOOCH_3$, base peak) characterize the amino acid valine.

Methylated metabolite A2 (A2-Me) shows a molecular ion M^+ at m/z 339. The mass spectrum suggests a conjugate of DJA with isoleucine as established by a comparison with authentic DJA-isoleucine. The fragmentation of A2-Me is similar to that of the valine conjugate (Table 1).

The CD curves of metabolites A1 and A2 showed negative Cotton effects as described for the (−)JA-isoleucine conjugate (Schneider et al. 1989), indicating that the (−)enantiomer of rac-DJA has been preferentially conjugated to give *N*-[(−)9,10-dihydrojasmonoyl]valine and *N*-[(−)9,10-dihydrojasmonoyl]isoleucine, respectively (Fig. 2).

The metabolite A3 differed from A2 by TLC in system 3 (A2, R_f = 0.68; A3, R_f = 0.60). The leucine part of the conjugate A3 could be confirmed by the MS data (Table 1). The mass spectrum of the leucine conjugate A3-Me shows a M^+ ion at m/z 339 similar to A2-Me but differs from the isoleucine derivative in the abundance ratio of ions at m/z 128 and m/z 131. In the case of A2-Me the fragment at m/z 128 (73%) is much more abundant than that at m/z 131 (38%). Contrary, in the leucine conjugate the peak of m/z 131 (88%) shows much higher intensity than that of m/z 128 (19%). This relationship is in accordance with the MS behavior of the synthetic isoleucine and leucine conjugates of JA, respectively (Schmidt et al. 1990). Thus, the metabolite A3 has been identified as *N*-[9,10-dihydrojasmonoyl]leucine.

The metabolites of group 2 (B1, B2a, and B2b) represent amino acid conjugates of DJA derivatives hydroxylated in the side chain. The structural elucidation was done by capillary GC-MS of the methylated and trimethylsilylated compounds. The mass spectrum of compound B1-Me-TMS shows a M^+ at m/z 413, corresponding to a valine conjugate of a hydroxylated DJA as established by the key ions **c, d,** $(c\text{-}COOCH_3)$, and $(d\text{-}HCOOCH_3)$ (Table 2, Fig. 3). Amino acid analysis confirmed valine as the amino acid moiety of B1. The presence of a hydroxylated DJA was indicated by the ions **b** and (**b**-TMSOH) in the MS of B1-Me-TMS. The hydroxy group is obviously located in the side chain, because ion **a** at m/z 255 is not shifted compared with that of the methylated metabolite A1, and its position at C-11 is deduced from the key ion $\mathbf{f_1}$ (CH_3-CH = O-TMS) at m/z 117 formed by an α-fission at C-11.

In summary metabolite B1 can be described as *N*-[11-hydroxy-9,10-dihydrojasmonoyl]valine.

The derivatized metabolites B2a and B2b gave both M^+ at m/z 427 corresponding to isoleucine conjugates of hydroxylated DJA. This is shown by the abundance ratio m/z 128 m/z 131 (see above). Furthermore, isoleucine was evidenced by amino

Table 3. Key ions of the mass spectra of amino acid conjugates with 2 hydroxyl groups, type III.

ion	B3a-Me m/z (relat. int. %)	B3b-ME m/z (relat. int. %)	ion	B3a-Me-TMS m/z (relat. int. %)	B3b-Me-TMS m/z (relat. int. %)
$M^{+\cdot}$	357 (1)	357 (2)	$M^{+\cdot}$	501 (5)	501 (7)
$[M\text{-}H_2O]^{+\cdot}$	339 (2)	339 (3)	$[M\text{-}\dot{C}H_3]^+$	486 (29)	486 (41)
m/z 313	(3)	(2)	$[M\text{-}59]^+$	—	442 (6)
$[M\text{-}59]^+$	298 (18)	298 (24)	$[M\text{-}TMSOH]^+$	411 (11)	411 (27)
$[M\text{-}59\text{-}H_2O]^+$	280 (12)	280 (17)	$[M\text{-}CH_3\text{-}TMSOH]^+$	396 (6)	396 (7)
$[M\text{-}59\text{-}2H_2O]^+$	262 (6)	—	m/z 370	(8)	(16)
c	187 (26)	187 (31)	e	357 (5)	357 (9)
d	146 (16)	146 (12)	m/z 354	(7)	(4)
m/z 135	(17)	(10)	$[M\text{-}TMSOH\text{-}CO_2Me]^+$	352 (6)	352 (13)
$(c\text{-}C_4H_8)$	131 (16)	131 (18)	$[M\text{-}2TMSOH]^+$	321 (20)	321 (13)
$(c\text{-}COOCH_3)$	128 (28)	128 (28)	m/z 282	(7)	(6)
$(d\text{-}HCOOCH_3)$	86 (100)	86 (100)	m/z 280	(7)	(8)
			(e-TMSOH)	267 (8)	267 (14)
			(e-TMSOH-H)	266 (7)	266 (16)
			m/z 265	(10)	(5)
			m/z 259	(14)	(9)
			m/z 250	(6)	(10)
			(b-TMSOH)	225 (29)	225 (9)
			m/z 203	(12)	(8)
			m/z 188	(21)	(48)
			c	187 (43)	187 (100)
			(e-2 TMSOH-H)	176 (21)	176 (21)
			m/z 155	(14)	(27)
			d	146 (40)	146 (67)
			(b-2TMSOH)	135 (43)	135 (51)
			$(c\text{-}C_4H_8)$	131 (32)	131 (62)
			$(c\text{-}\dot{C}OOCH_3)$	128 (47)	128 (90)
			f	117 (40)	103 (13)
			$(d\text{-}HCOOCH_3)$	86 (54)	86 (78)
			m/z 75	(47)	(50)
			$[TMS]^+$	73 (100)	73 (90)

acid analysis. The MS fragmentation pattern of both B2a-Me-TMS and B2b-Me-TMS is similar to that described for the valine derivative B1-Me-TMS. In the B2a-Me-TMS spectrum the appearance of the fragment at m/z 117 ($\mathbf{f_1}$) suggests a hydroxylation at C-11, and thus, the structure is established to be *N*-[11-hydroxy-9,10-dihydrojasmonoyl]isoleucine. Contrary, B2b-Me-TMS shows a **f**-type ion at m/z 103 ($\mathbf{f_2}$), originating by an α-fission at C-12 (CH_2 = O-TMS; see Meyer et al. 1989). Therefore, metabolite B2b is characterized to be *N*-[12-hydroxy-9,10-dihydrojasmonoyl]isoleucine. The ratio of the C-11-hydroxylated isoleucine conjugate (B2a) to the C-12-hydroxylated one (B2b) is about 2:1 as calculated from GC data.

Group 3 (metabolites B3a and B3b; Fig. 2) represents isoleucine conjugates in which the side chain of the DJA moiety is hydroxylated and the keto function of the cyclopentane ring is reduced to a hydroxyl group. The characterization was performed by a combination of capillary GC-MS of the methylated and GC-MS of the additionally trimethylsilylated compounds. The MS data of both types of spectra are summarized in Table 3. B3a-Me and B3b-Me, separated by GC gave the same M^+ at m/z 357. Some key fragments [**c**, **d**, ($\mathbf{c}\text{-}C_4H_8$), ($\mathbf{c}\text{-}COOCH_3$), and ($\mathbf{d}\text{-}HCOOCH_3$)] suggest these metabolites to be isoleucine conjugates (Fig. 3). After trimethylsilylation of B3-Me two components (B3a-Me-TMS and B3b-Me-TMS) with the same M^+ at m/z 501 are found. The M^+ and the fragment $[M\text{-}2TMSOH]^+$ indicate the original presence of two hydroxyl groups in the metabolites B3a and B3b. One hydroxyl group is located in the side chain as evidenced by the appearance of **f**-type ions at m/z 117 (B3a-Me-TMS) and m/z 103 (B3b-Me-TMS). Obviously, the second hydroxyl group is attached to position 6 of the ring system. The stereochemistry of the hydroxyl group at C-6 remained open. Therefore, the metabolites B3a and B3b can be described as the cucurbic acid-related compounds *N*-{[3-hydroxy-2-(4-hydroxypentyl)-cyclopent-1-yl]-acetyl}isoleucine (*N*-[6-deoxy-6,11-dihydroxy-9,10-dihydrojasmonoyl]isoleucine) and *N*-{[3-hydroxy-2-(5-hydroxypentyl)-cyclopent-1-yl]-acetyl}isoleucine (*N*-[6-deoxy-6,12-dihydroxy-

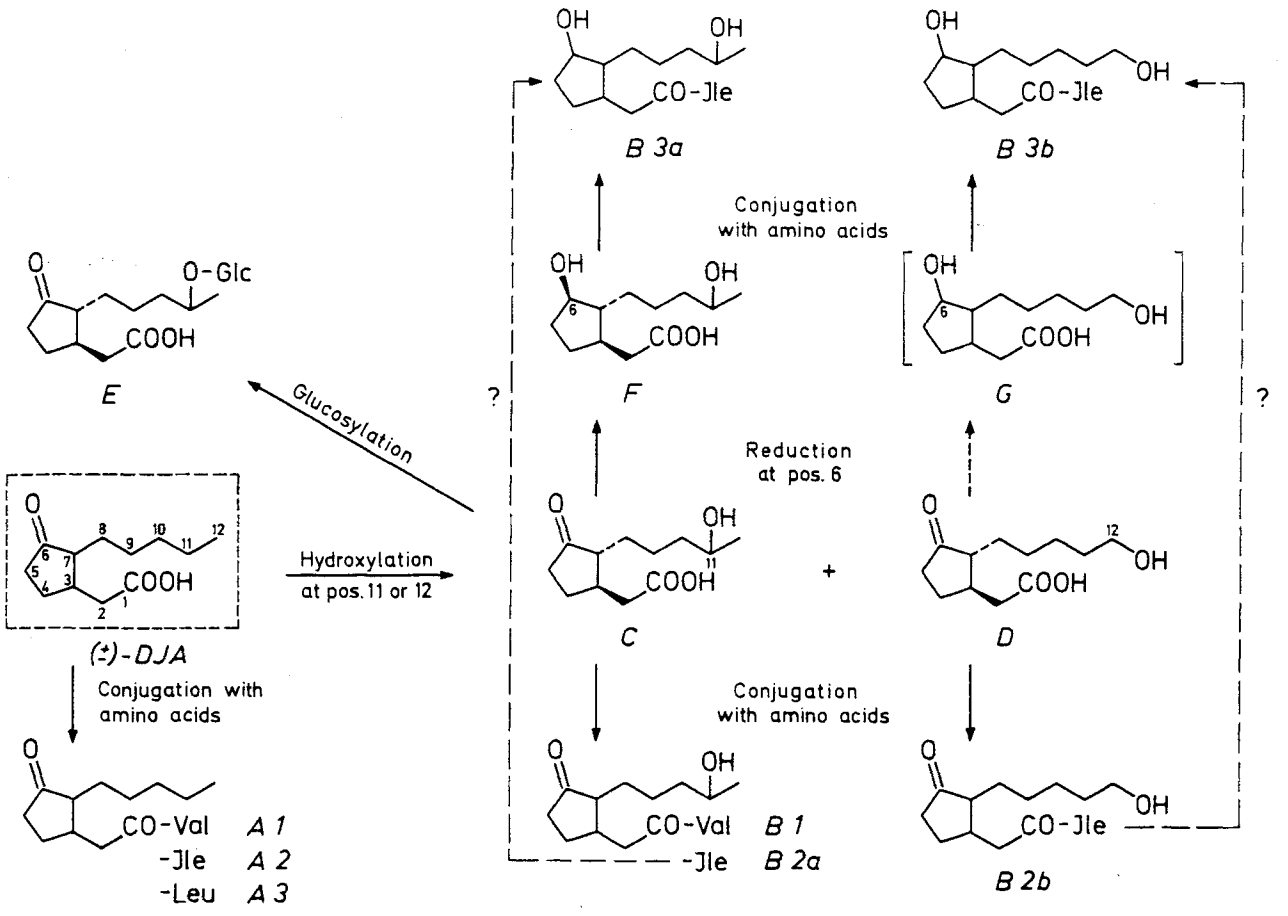


Fig. 4. Proposed metabolism of (±)9,10-DJA in barley shoots.

9,10-dihydrojasmonoyl]isoleucine), respectively (see Fig. 2). Their ratio of about 4:1 was determined by GC.

Discussion

A survey on the metabolism of (±)DJA in barley shoots is given in Fig. 4. As described recently (Meyer et al. 1989), the most important step in the metabolism of (±)DJA and also of JA (data not shown) in barley seedlings seems to be the hydroxylation at position 11 followed by glucosylation (see metabolites C and E in Fig. 4).

We present herein another kind of metabolite formed from (±)DJA in barley shoots by conjugation with amino acids, especially valine and isoleucine. These minor metabolites have been structurally elucidated by GC-MS and belong to three types of conjugates (Fig. 2). Group 1 derives from DJA which is bound to valine, isoleucine, and leucine giving *N*-[(−)9,10-dihydrojasmonoyl]valine (A1), *N*-[(−)9,10-dihydrojasmonoyl]isoleucine (A2), and *N*-[9,10-dihydrojasmonoyl]leucine (A3). Group 2 formally arises from 11-OH-DJA or 12-OH-DJA bound to valine or isoleucine resulting in *N*-[11-hydroxy-9,10-dihydrojasmonoyl]valine (B1), *N*-[11-hydroxy-9,10-dihydrojasmonoyl]isoleucine (B2a), and *N*-[12-hydroxy-9,10-dihydrojasmonoyl]-isoleucine (B2b). Group 3 formally comes from 11-OH-DJA or 12-OH-DJA which is additionally reduced at the keto group and conjugated with isoleucine to give *N*-{[3-hydroxy-2-(4-hydroxypentyl)-cyclopent-1-yl]-acetyl}isoleucine (B3a) and *N*-{[3-hydroxy-2-(5-hydroxypentyl)-cyclopent-1-yl]-acetyl}isoleucine (B3b), which are structurally related to 7-iso-cucurbic acid.

It was assumed that the amino acid conjugates mainly contain components with a *trans* configuration of the side chain at C-7 similar to the applied (±)DJA. But because racemic DJA applied to barley seedlings contains also a small amount of iso-DJA (*cis* configuration of the side chains, Fig. 1) the formation of conjugates with *cis* configuration to a certain degree can not be excluded.

Obviously in barley shoots only aliphatic amino acids have been involved in the conjugation. Con-

jugates of this type are formed in *Gibberella fujikuroi*, i.e., the *N*-[jasmonoyl]- and *N*-[dihydrojasmonoyl]isoleucine (Cross and Webster 1970), whereas the *N*-[(−)jasmonoyl]-S-isoleucine is found to occur naturally in apical leaves and young fruits of *Vicia faba* (Schmidt et al. 1990, Schneider et al. 1989). However, metabolites conjugated with aromatic amino acids like the *N*-(12-acetoxyjasmonoyl)phenylalanine methyl ester from *Praxelis clematidea* (Bohlmann et al. 1984) or the *N*-[(−)jasmonoyl]-S-tyrosine and *N*-[(−)jasmonoyl]-S-tryptophan from flowers of *Vicia faba* (Brückner et al. 1986, Brückner et al. 1988) could not be detected in barley shoots.

As mentioned above, in the metabolism of DJA the hydroxylation seems to be a very important step. It leads to the main metabolite 11-hydroxy-9,10-DJA (C) and its O(11)-β-D-glucopyranoside (E) and to a lesser extent to 12-hydroxy-9,10-DJA (D) (Fig. 4) (Meyer et al. 1989). The hydroxyl group does not only occur in the free acids but also in the amino acid conjugates. Whether hydroxylation is the first step which is followed by conjugation to amino acids or vice versa is not yet clear. Obviously, in the metabolism of DJA and also JA (data not shown) hydroxylation in position 11 is favored in relation to position 12. This could be demonstrated for the hydroxydihydrojasmonic acids, where a ratio of 11-OH-DJA/12-OH-DJA = 9:1 was found. Similar results were obtained for the amino acid conjugates with ratios of 6:4 and 8:2. But as endogenous compounds derivatives of C-12-hydroxylated JA, such as the jasmine ketolactone in *Jasminum grandiflorum* (Demole et al. 1964), the *N*-(12-acetoxyjasmonoyl)-phenylalanine methyl ester in *Praxelis clematidea* (Bohlmann et al. 1984), and the 12-hydroxyjasmonic acid O(12)-β-D-glucopyranoside as the tuber-inducing substance in potato (Yoshihara et al. 1988, 1989) are found. Further metabolic steps are the reduction at position 6 (without conjugation to amino acids), resulting in substances (F, Fig. 4) related to cucurbic acid (Fukui et al. 1977a,b), and the glucosylation of the hydroxyl groups or the formation of glucosyl esters, which will be described elsewhere.

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