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SYNTHESIS OF N-(JASMONOYL)AMINO ACID CONJUGATES

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Abstract - Both racemic jasmonic acid [(±)-JA] and the naturally occurring (-)-enantiomer [(-)-JA] have been reacted with aliphatic, aromatic as well as acidic amino acids to form amide-linked derivatives. The diastereoisomeric products of (±)-jasmonic acid with S-Val, S-Leu, S-Ile, S-Phe, S-Trp, R-Val, and R-Phe could be separated by silica gel chromatography. The synthesized N-(jasmonoyl)-conjugates have been structurally characterized by MS, ^{1}H NMR, IR and ORD.

Introduction

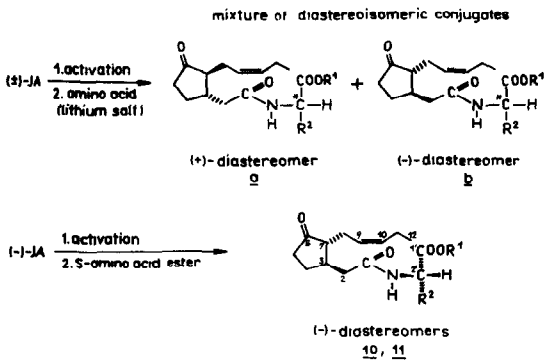
Investigations on the occurrence of jasmonic acid and its methyl ester (JA-Me) have demonstrated that they are widely distributed in plants [1-5]. This new type of plant growth regulators possesses different biological activities when exogenously applied to plants [6-9]. Furthermore, amino acid conjugates of JA and of related compounds have been shown to occur naturally [10-13]. In order to study biochemical and physiological aspects of the JA conjugation, the availability of N-(jasmonoyl)-amino acids is necessary.

Phytohormones carrying a carboxylic function, e. g. indole-3-acetic acid and gibberellins, are known to be derivatized with compounds containing amino groups by promotion with alkyl chlorocarbonates and carbodiimides [14-17]. Isobutyl chloroformate, one of the most powerful reagents in the synthesis of amide bonds, was used to activate both racemic JA and its native (-)-form. In the following the synthesis and separation of diastereoisomeric N-(jasmonoyl)-conjugates of (S)- and (R)-amino acids as well as their structural characterization are described.

Results and Discussion

Racemic JA was obtained by alkaline hydrolysis of synthetic (±)-JA-Me and subsequent chromatography with solvent A. Purified (±)-JA was activated with isobutyl chloroformate and triethylamine in tetrahydrofuran at -10° C for 1 h. The aminolysis of the resulting mixed anhydride with the lithium salts of S-Val, S-Leu, S-Ile, S-Phe, S-Trp, R-Val, R-Phe, S-Asp, and S-Glu in aqueous THF at 4° C for 5 h afforded a mixture of the diastereoisomeric conjugates of the a and b type (Fig. 1). Enantiomerically pure (-)-JA was activated by the same procedure followed by the aminolysis with S-alanine methyl ester and

S-tyrosine ethyl ester in the presence of triethylamine to give the corresponding conjugates 10 and 11 (Fig. 1).



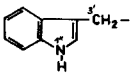
		R^1	R^2
1a 1b	N-[(+)-jasmonoyl]-S-valine N-[(-)-jasmonoyl]-S-valine	H	$-^{3'}CH(CH_3)_2$
2a 2b	N-[(+)-jasmonoyl]-S-leucine N-[(-)-jasmonoyl]-S-leucine	H	$-^{4'}CH_2-^{3'}CH(CH_3)_2$
3a 3b	N-[(+)-jasmonoyl]-S-isoleucine N-[(-)-jasmonoyl]-S-isoleucine	H	$-^{3'}CH(CH_3)-^{4'}CH_2-^{5'}CH_3$
4a 4b	N-[(+)-jasmonoyl]-S-phenylalanine N-[(-)-jasmonoyl]-S-phenylalanine	H	$-^{3'}CH_2-C_6H_5$
5a 5b	N-[(+)-jasmonoyl]-S-tryptophan N-[(-)-jasmonoyl]-S-tryptophan	H	
6a 6b	N-[(+)-jasmonoyl]-R-phenylalanine N-[(-)-jasmonoyl]-R-phenylalanine	H	$-^{3'}CH_2-C_6H_5$
7a 7b	N-[(+)-jasmonoyl]-R-valine N-[(-)-jasmonoyl]-R-valine	H	$-^{3'}CH(CH_3)_2$
8	N-[(±)-jasmonoyl]-S-aspartic acid	H	$-^{3'}CH_2-^{4'}COOH$
9	N-[(±)-jasmonoyl]-S-glutamic acid	H	$-^{3'}CH_2-^{4'}CH_2-^{5'}COOH$
10	N-[(-)-jasmonoyl]-S-alanine methyl ester	$-CH_3$	$-^{3'}CH_3$
11	N-[(-)-jasmonoyl]-S-tyrosine ethyl ester	$-CH_2CH_3$	$-^{3'}CH_2-C_6H_4-OH$

Fig. 1: Synthesis of diastereoisomeric N-(jasmonoyl)-amino acid conjugates

The diastereoisomeric mixture of each conjugate obtained after evaporation of the organic solvent and partitioning the aqueous concentrate at pH 3-4 with chloroform were applied onto column chromatography with silica gel. By repeated chromatography a separation of the N-[(+)-jasmonoyl]-7- and the N-[(-)-jasmonoyl]-7-amino acid conjugates 1a/b - 7a/b was acchieved using solvent B. Under these chromatographic conditions, the (+)-diastereomers 1a, 2a, 3a, 4a, and 5a were eluted before the (-)-diastereomers 1b, 2b, 3b, 4b, and 5b. The elution behaviour of the diastereoisomeric compounds 6a/b and 7a/b was found to be reverse. The (±)-JA conjugates 8 and 9 with the acidic amino acids S-Asp and S-Glu, respectively, were purified by silica gel chromatography using solvent D. The N-[(-)-jasmonoyl]-7-S-amino acid esters 10 and 11 were chromatographed on silica gel with solvent systems C and E. The purified and

separated compounds were crystallized from either chloroform-n-hexane (1a, 1b, 2a, 2b, 3a, 3b, 7a, 7b, 10) or ethyl acetate-n-hexane (4a, 4b, 5a, 5b, 6a, 6b), see Table 1.

The efficiency of the chromatographic separation on silica gel and the optical purity of the products were checked by reverse-phase HPLC [18] (see Tab. 1 and Fig. 2).

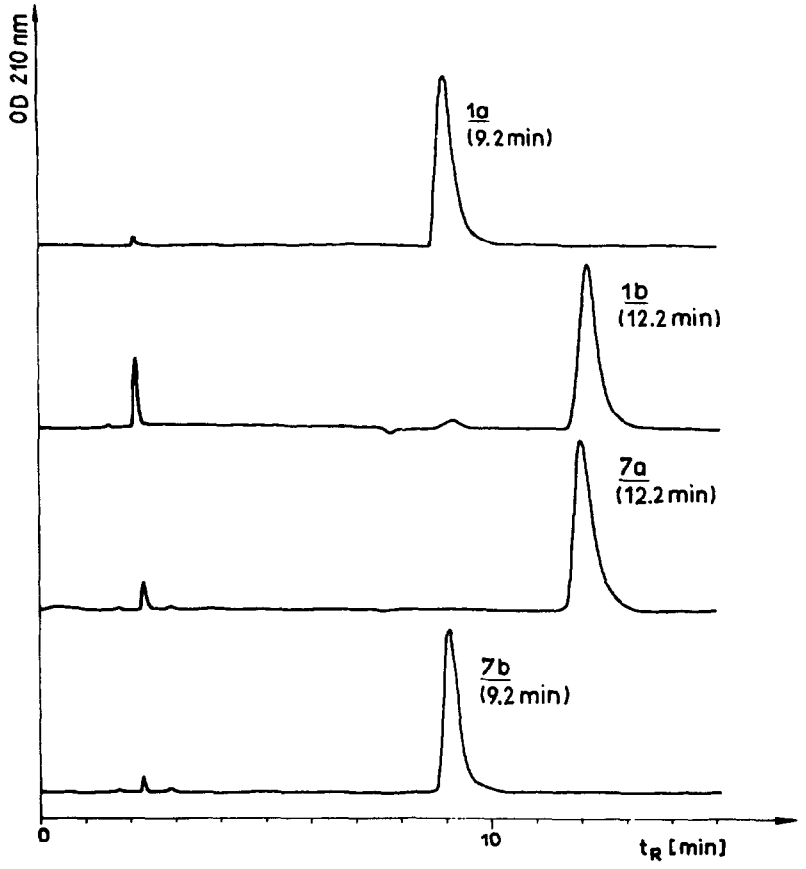


Fig. 2: HPLC of diastereoisomeric N-jasmonoyl-conjugates (see Experimental)

In order to confirm the stereochemistry of the JA moiety of the synthesized conjugates the optical rotary dispersion has been measured in methanol. As expected, all (-)-JA conjugates show a negative Cotton effect with extrema at 314-316 nm and 268-276 nm for the carbonyl chromophore (295-300 nm), whereas the (+)-JA conjugates are characterized by a corresponding positive Cotton effect with the same extrema (see Tab. 1).

The mass spectral fragmentation of the synthetic N-(jasmonoyl)-amino acid derivatives 1a-11 is shown in figure 3.

As given in Tab. 2, the positive ion mass spectra [19] (10-16 eV) of all compounds investigated yield a $[M\_7]^{+\cdot}$- ion together with fragments originating from $[M-H_2O\_7]^{+\cdot}$- and $[M-HCO_2R\_7]^{+\cdot}$-ions. The fragment ions a, (b-H), (e+H), (c-H), and i/(i+H) comprise the jasmonic acid moiety, whereas the ions at (f+H), (h+2H), (k-H), m, and (n+H) contain the amino acid residue.

Table 1: Properties of diastereoisomeric N-(jasmonoyl)-amino acid conjugates

compound	m. p. (° C)	$[\alpha]_D$ (° C)	molecular rotation $[\Phi_1]$ (314 - 316 nm)	molecular rotation $[\Phi_2]$ (268 - 276 nm)	Cotton effect[3]	c (g/100 ml)	HPLC t_R (min)
1a	105 - 107	+41.1° (23)	+2290°	-2883°	pos.	0.27	9.2
1b	140 - 142	-55.5° (24)	-2450°	+2756°	neg.	0.28	12.2
2a	134 - 136	+33.1° (23)	+1482°	-1875°	pos.	0.26	15.6
2b	145 - 147	-64.9° (25)	-1736°	+1935°	neg.	0.23	22.2
3a	135 - 137	+48.8° (24)	+1705°	-1705°	pos.	0.27	16.0
3b	150 - 152	-45.2° (23)	-1309°	+1955°	neg.	0.28	22.4
4a	103 - 106	+45.1° (24)	+1699°	-1514°	pos.	0.25	17.8
4b	163 - 165	-30.5° (23)	-1416°	+2222°	neg.	0.31	22.4
5a	101 - 103	+30.7° (24)[1]	+1692°	-1908°	pos.	0.022	16.3
5b	149 - 150	-32.1° (25)[2]	-1520°	+2344°	neg.	0.025	18.4
6a	157 - 160	+27.3° (23)	+1885°	-3838°	pos.	0.21	22.5
6b	103 - 106	-45.5° (24)	-2467°	+2531°	neg.	0.22	17.7
7a	135 - 137	+46.9° (22)	+1465°	-2126°	pos.	0.21	12.2
7b	105 - 108	-23.7° (22)	-1446°	+1560°	neg.	0.22	9.2
10	95 - 98	-84.6° (23)	-1336°	+1717°	neg.	0.22	-
11	oil	-34.9° (23)	-2935°	+3914°	neg.	0.29	-

[1] c = 0.364 [2] c = 0.372 [3] Cotton effect of the carbonyl chromophore

Table 2: Key ions in the positive ion mass spectra (10 - 16 eV) of N-(jasmonoyl)-amino acid conjugates [m/z(rel. int., %)]

ion	1a	1b	2a	2b	3a	3b	7a	7b
$[M]^{+\bullet}$	309 (54)	309 (45)	323 (61)	323 (73)	323 (54)	323 (50)	309 (42)	309 (60)
$[M-H_2O]^{+\bullet}$	291 (43)	291 (42)	305 (42)	305 (48)	305 (33)	305 (30)	291 (20)	291 (42)
$[M-CO_2R^1]^+$	264 (17)	264 (16)						
$[M-HCO_2R^1]^{+\bullet}$			277 (18)	277 (13)	277 (9)	277 (12)		263 (14)
$[M-HCO_2R^1-C_3H_7]^+$			234 (13)	234 (11)				
a	151 (51)	151 (66)	151 (69)	151 (75)	151 (54)	151 (65)	151 (44)	151 (73)
(b-H)	164 (36)	164 (37)	164 (32)	164 (35)	164 (25)	164 (33)	164 (17)	164 (37)
(c-H)	192 (36)	192 (33)	192 (38)	192 (37)	192 (24)	192 (29)	192 (16)	192 (37)
d/(d-H)	208 (11)	207 (20)	208 (26)	207 (18)	208 (12)	208 (11)	208 (5)	208 (12)
(e+H)	241 (36)	241 (35)	255 (32)	255 (39)	255 (24)	255 (26)	241 (14)	241 (37)
(f+H)	159 (89)	159 (86)	173 (82)	173 (88)	173 (78)	173 (88)	159 (75)	159 (96)
(h+2H)	118 (85)	118 (86)	132 (90)	132 (95)	132 (80)	132 (89)	118 (61)	118 (98)
i/(i+H)			267 (10)	267 (11)	266 (8)	267 (12)		
(i/i+H-H_2O)			249 (55)	249 (11)	248 (18)	249 (9)		
(f+H-CO_2R^1)	114 (80)	114 (80)	128 (61)	128 (65)	128 (63)	128 (77)	114 (59)	114 (93)
(f-C_4H_8)			117 (92)	117 (97)	117 (67)	117 (82)		
(h+2H-HCO_2R^1)	72 (100)	72 (100)	86 (100)	86 (100)	86 (100)	86 (100)	72 (100)	72 (100)

Table 2: continued

ion	4a	4b	5a	5b[a]	6a	6b	11
$[M]^{+\cdot}$	357 (54)	357 (58)	396 (3)	410 (23)	357 (47)	357 (36)	401 (41)[e]
$[M-H_2O]^{+\cdot}$	339 (74)	339 (54)	378 (25)	378 (21)	339 (37)	339 (28)	
$[M-HCO_2R^1]^{+\cdot}$	311 (35)	311 (18)	350 (2)	351 (3)	311 (10)	311 (7)	
a	151 (48)	151 (62)	151 (5)	151 (10)	151 (58)	151 (50)	
(c-H)	192 (39)	192 (38)			192 (80)	192 (26)	192 (100)
(e+H)	289 (19)	289 (35)			289 (22)	289 (16)	
(f+H)	207 (57)	207 (84)			207 (75)	207 (70)	251 (35)
(h+2H)	166 (58)	166 (85)			166 (87)	166 (78)	210 (63)
(k-H)	148 (84)	148 (100)	187 (9)	201 (65)	148 (100)	148 (100)	192 (100)
(i/i+H-H_2O)	248 (100)	248 (41)			248 (25)	248 (30)	
m	91 (89)	91 (75)	130 (100)	130 (100)	91 (57)	91 (58)	107 (96)
(n+H)			117 (8)	117 (9)			
(f+H-CO_2)	163 (61)	163 (57)			162 (41)[b]	162 (35)[b]	
(h+2H-HCO_2R^1)	120 (93)	120 (92)			120 (93)	120 (93)	136 (76)
(k-H-CO_2)	104 (84)	104 (62)	143 (15)	143 (19)	104 (39)	104 (32)	

Table 2: continued

ion	8[c]	9[d]	10
$[M]^{+\cdot}$	353 (21)	367 (22)	295 (26)
$[M-H_2O]^{+\cdot}$	335 (9)	349 (9)	277 (9)
a	151 (25)	151 (36)	151 (23)
(c-H)	192 (11)	192 (32)	192 (12)
(e+H)	285 (7)	299 (12)	227 (11)
(f+H)	203 (94)	217 (100)	145 (93)
(h+2H)	162 (89)	176 (72)	104 (100)
(k-H)	144 (37)	158 (57)	86 (66)
$(f+H-CO_2R^1)$	144 (37)	158 (57)	86 (66)
$(h+2H-HCO_2R^1)$	102 (100)	116 (84)	

[a] methyl ester; m/z 378 $[M-MeOH]^{+\cdot}$, m/z 351 $[M-CO_2Me]^{+}$, m/z 143 $(k-CO_2Me)$

[b] m/z 162 $(f+H-CO_2H)$

[c] dimethyl ester

[d] dimethyl ester

[e] $[M+1]^{+}$ m/z 402 (54)

Table 3: Key ions in the negative ion mass spectra (2 - 4 eV) of N-(jasmonoyl)-amino acid conjugates [m/z (rel. int. %)]

ion	1a	1b	2a	2b	3a	3b	7a	7b
$[M-1]^-$	308 (48)	308 (14)	322 (86)	322 (47)	322 (100)	322 (100)	308 (43)	308 (85)
$[M-HCO_2R^1]^{-\bullet}$			277 (15)	277 (15)	277 (7)	277 (8)	263 (15)	263 (22)
$[M-HCO_2R^1-C_3H_7]^-$			234 (9)	234 (8)				
$[b]^-$	165 (20)	165 (16)	165 (18)	165 (23)			165 (10)	165 (15)
$[d/d+H]^-$	209 (16)	209 (23)		208 (5)				
$[e]^-$	240 (31)	240 (13)	254 (35)	254 (22)	254 (10)	254 (16)	240 (20)	240 (29)
$[h]^-$			130 (21)	130 (27)	130 (39)	130 (22)		
$[h-2H]^-$	114 (100)	114 (100)	128 (100)	128 (100)	128 (18)	128 (18)	114 (100)	114 (100)
$[i/i-H]^-$	266 (9)	266 (14)						
$[k-H]^{-\bullet}$	100 (22)	100 (10)	114 (30)	114 (29)	114 (21)	114 (28)	100 (16)	100 (49)
$[f-H_2O]^-$	140 (13)	140 (21)						

Table 3: continued

ion	4a	4b	5a	5b[a]	6a	6b	11[d]
$[M-1]^-$	356 (46)	356 (51)	395 (22)	409 (41)	356 (100)	356 (100)	400 (49)
$[M-HCO_2R^1]^{-\bullet}$	311 (80)	311 (100)	350 (100)	350 (12)	311 (15)	311 (8)	
$[M-HCO_2R^1-C_8H_5N]^-$			234 (67)	234 (20)			
$[a-H]^-$			150 (19)	150 (24)			
$[e]^-$	288 (15)	288 (35)	327 (13)		288 (25)	288 (16)	332 (12)
$[h]^-$ / $[h+H]^{-\bullet}$	165 (27)	165 (21)			164 (37)	164 (27)	
$[i/i-H]^-$	266 (18)	266 (21)	266 (11)	279 (63)		266 (33)	293 (27)
$[i/i-H-H_2O]^-$	248 (100)	248 (38)	248 (17)	248 (40)	248 (10)	248 (7)	248 (19)
$[k-2H]^-$	147 (29)	147 (12)		200 (92)			191 (46)
$[m-H]^-$			129 (38)	129 (95)			106 (62)
$[n]^-$			116 (11)	116 (34)			
$[k-H-CO_2R^1]^-$	103 (12)	103 (7)	142 (15)	142 (100)	103 (8)	103 (8)	

Table 3: continued

ion	8[b]	9[c]	10	
$[M-1]^-$	352 (39)	366 (18)	294 (44)	
$[M-HCO_2R^1]^{-\bullet}$	293 (31)	307 (13)		[a] methyl ester; m/z 248 $[i-H-MeOH]^-$
$[M/M-1-MeOH]^-$	321 (10)	334 (18)	262 (5)	[b] dimethyl ester
$[M-HCO_2R^1-CH_2CO_2R^1]^-$		234 (52)		[c] dimethyl ester
$[d]^-$	208 (18)	208 (19)	208 (33)	[d] base peak at m/z 98 $[f-EtOH-CH_2C_6H_4O]^-$
$[e]^-$			226 (6)	
$[h]^-$	160 (18)			
$[i-H]^-$	279 (100)	279 (5)		
$[k-H]^-$	144 (42)		186 (38)	
$[f-MeOH]^-$		184 (11)	112 (100)	
$[f-2MeOH]^-$		152 (10)		
$[f+H-CH_3CO_2R^1]^-$	129 (44)			
$[f+H-CH_2CHCO_2R^1]^-$		131 (100)		
$[h-MeOH]^-$		142 (19)		
$[i-H-MeO]^-$	248 (45)	248 (5)		
$[k-H-MeOH]^-$	112 (30)			

Table 4: Selected proton n. m. r. data[a] of N-(jasmonoyl)-amino acid conjugates

proton	1a	1b	2a	2b	3a	3b
$C(12)H_3$	t, 0.96 ($J_{12,11}$ 7.3)	t, 0.97 ($J_{12,11}$ 7.5)	t, 0.97 ($J_{12,11}$ 7.5)	t, 0.97 ($J_{12,11}$ 7.5)	t, 0.96 ($J_{12,11}$ 7.5)	t, 0.94 ($J_{12,11}$ 7.5)
C(9)H, C(10)H	2 br m 5.2 - 5.5	2 br m 5.2 - 5.5	2 br m 5.3 - 5.5	2 br m 5.3 - 5.5	2 br m 5.2 - 5.5	2 br m 5.2 - 5.5.
$C(1')OCH_3$	s, 3.75	s, 3.75	s, 3.74	s, 3.74	s, 3.75	s, 3.72
C(2')NH	br d, 5.95 ($J_{NH,2'}$ 8.7)	br d, 5.92 ($J_{NH,2'}$ 8.7)	br d, 5.82 ($J_{NH,2'}$ 8.5)	br d, 5.85 ($J_{NH,2'}$ 8.5)	br d, 5.90 ($J_{NH,2'}$ 8.6)	br d, 5.92 ($J_{NH,2'}$ 8.5)
C(2')H	dd, 4.60 ($J_{2',NH}$ 8.7) ($J_{2',3'}$ 4.8)	dd, 4.58 ($J_{2',NH}$ 8.7) ($J_{2',3'}$ 4.8)	m, 4.67	m, 4.65	dd, 4.64 ($J_{2',NH}$ 8.6) ($J_{2',3'}$ 4.8)	dd, 4.59 ($J_{2',NH}$ 8.5) ($J_{2',3'}$ 4.8)
	$C(3')H(\underline{CH_3})_2$ 2xd, 0.91, 0.95 (J 8.0)	$C(3')H(\underline{CH_3})_2$ 2xd, 0.91, 0.95 (J 7.8)	$C(4')H(\underline{CH_3})_2$ d, 0.95 (J 5.2)	$C(4')H(\underline{CH_3})_2$ d, 0.95 (J 5.3)	$C(3')H\underline{CH_3}$ d, 0.91 (J 7.0) $C(5')H_3$ t, 0.93 (J 7.0)	$C(3')H\underline{CH_3}$ d, 0.88 (J 6.8) $C(5')H_3$ t, 0.90 (J 6.8)

[a] the chemical shifts are given in ppm downfield from TMS; the vicinal coupling constants (J) are obtained in Hz.

Table 4: continued

proton	4a	4b	5a	5b	6a	6b
C(12)H_3	t, 0.87 ($J_{12,11}$ 7.5)	t, 0.93 ($J_{12,11}$ 7.5)	t, 0.95 ($J_{12,11}$ 7.5)	t, 0.93 ($J_{12,11}$ 7.5)	t, 0.93 ($J_{12,11}$ 7.5)	t, 0.92 ($J_{12,11}$ 7.5)
C(9)H, C(10)H	2 br m 5.2 - 5.4	2 br m 5.2 - 5.4	2 br m 5.2 - 5.4	2 br m 5.2 - 5.4	2 br m 5.2 - 5.4	2 br m 5.2 - 5.4
C(1')OCH_3	s, 3.68	s, 3.73	s, 3.72	s, 3.73	s, 3.73	s, 3.73
C(2')NH	br d, 5.79 ($J_{NH,2'}$ 7.8)	br d, 5.84 ($J_{NH,2'}$ 7.8)	br d, 5.93 ($J_{NH,2'}$ 7.9)	br d, 5.92 ($J_{NH,2'}$ 7.9)	br d, 5.81 ($J_{NH,2'}$ 7.7)	br d, 5.83 ($J_{NH,2'}$ 7.7)
C(2')H	dt, 4.86 ($J_{2',NH}$ 7.8) ($J_{2',3'a}$ 6.3) ($J_{2',3'b}$ 5.7)	dt, 4.88 ($J_{2',NH}$ 7.8) ($J_{2',3'a}$ 6.3) ($J_{2',3'b}$ 5.7)	dt, 4.98 ($J_{2',NH}$ 7.9) ($J_{2',3'a}$ = $J_{2',3'b}$ 5.4)	dt, 4.98 ($J_{2',NH}$ 7.9) ($J_{2',3'a}$ = $J_{2',3'b}$ 5.5)	dt, 4.89 ($J_{2',NH}$ 7.7) ($J_{2',3'a}$ 6.3) ($J_{2',3'b}$ 5.7)	dt, 4.91 ($J_{2',NH}$ 7.7) ($J_{2',3'a}$ 6.3) ($J_{2',3'b}$ 5.7)
	C(3')H_a dd, 3.01 ($J_{3'a,2'}$ 6.3) ($J_{3'a,3'b}$ 13.9)	C(3')H_a dd, 3.05 ($J_{3'a,2'}$ 6.3) ($J_{3'a,3'b}$ 13.9)	C(3')H_a dd, 3.30 ($J_{3'a,2'}$ 5.4) ($J_{3'a,3'b}$ 14.5)	C(3')H_a dd, 3.30 ($J_{3'a,2'}$ 5.5) ($J_{3'a,3'b}$ 14.6)	C(3')H_a dd, 3.05 ($J_{3'a,2'}$ 6.3) ($J_{3'a,3'b}$ 13.9)	C(3')H_a dd, 3.06 ($J_{3'a,2'}$ 6.3) ($J_{3'a,3'b}$ 13.9)
	C(3')H_b dd, 3.12 ($J_{3'b,2'}$ 5.7) ($J_{3'b,3'a}$ 13.9)	C(3')H_b dd, 3.15 ($J_{3'b,2'}$ 5.7) ($J_{3'b,3'a}$ 13.9)	C(3')H_b dd, 3.36 ($J_{3'b,2'}$ 5.4) ($J_{3'b,3'a}$ 14.5)	C(3')H_b dd, 3.35 ($J_{3'b,2'}$ 5.5) ($J_{3'b,3'a}$ 14.6)	C(3')H_b dd, 3.15 ($J_{3'b,2'}$ 5.7) ($J_{3'b,3'a}$ 13.9)	C(3')H_b dd, 3.16 ($J_{3'b,2'}$ 5.7) ($J_{3'b,3'a}$ 13.9)
	aromat. H_5 2 m, 7.02, 7.2	aromat. H_5 2 m, 7.1, 7.3	indole H_5 6.95 - 7.6 indole NH br s, 8.13	indole H_5 6.95 - 7.6 indole NH br s, 8.12	aromat. H_5 2 m, 7.05, 7.35	aromat. H_5 2 m, 7.0, 7.3

Table 4: continued

proton	7a	7b	8	9	10	11
$C(12)H_3$	t, 0.94 ($J_{12,11}$ 7.5)	t, 0.94 ($J_{12,11}$ 7.5)	t, 0.94 ($J_{12,11}$ 7.5)	t, 0.89 ($J_{12,11}$ 7.5)	t, 0.95 ($J_{12,11}$ 7.5)	t, 0.94 ($J_{12,11}$ 7.5)
C(9)H, C(10)H	2 ... m 5.2 - 5.5	2 br m 5.2 - 5.5	2 br m 5.3 - 5.5	2 br m 5.2 - 5.4	2 br m 5.3 - 5.45	2 br m 5.2 - 5.4
$C(1')OCH_3$	s, 3.73	s, 3.73	s, 3.75[b]	s, 3.69[b]	s, 3.77	-
C(2')NH	br d, 5.89 ($J_{NH,2'}$ 8.8)	br d, 5.91 ($J_{NH,2'}$ 8.7)	br d, 6.48 ($J_{NH,2'}$ 8.0)	br s, 6.23	br s, 6.02	br d, 5.87 ($J_{NH,2'}$ 7.9)
C(2')H	dd, 4.56 ($J_{NH,2'}$ 8.8) ($J_{2',3'}$ 4.8)	dd, 4.58 ($J_{NH,2'}$ 8.7) ($J_{2',3'}$ 4.8)	2xdt, 4.85, 4.86 ($J_{NH,2'}$ 8.0) ($J_{2',3'a}$ 4.1) ($J_{2',3'b}$ 4.3)	m, 4.56	dq, 4.61 ($J_{NH,2'}$ = $J_{2',3'}$ 7.2)	dt, 4.84 ($J_{NH,2'}$ 7.9) ($J_{2',3'a}$ 5.8) ($J_{2',3'b}$ 6.2)
	$C(3')H(CH_3)_2$ 2xd, 0.89, 0.92 (J 6.9)	$C(3')H(CH_3)_2$ 2xd, 0.88, 0.92 (J 6.9)	$C(3')H_a$ 2xdd, 2.82, 2.83 ($J_{3'a,2'}$ 4.1) ($J_{3'a,3'b}$ 17.3)	$C(5')OCH_3$ s, 3.62[b]	$C(3')H_3$ d, 1.43 ($J_{3',2'}$ 7.2)	$C(3')H_a$ dd, 2.98 ($J_{3'a,2'}$ 5.8) ($J_{3'a,3'b}$ 14.0)
			$C(3')H_b$ dd, 3.04 ($J_{3'b,2'}$ 4.3) ($J_{3'b,3'a}$ 17.3)			$C(3')H_b$ dd, 3.08 ($J_{3'b,2'}$ 6.2) ($J_{3'b,3'a}$ 14.0)
			$C(4')OCH_3$ s, 3.68[b]			$C(1')OCH_2CH_3$ q, 4.18 (J 7.1)
						$C(1')OCH_2CH_3$ t, 1.26 (J 7.1)
						aromat. H_4 2x d, 6.72, 6.95 (J 8.5)

[b] assignment may be interchanged

On the contrary, the negative ion mass spectra (2-4 eV)[20] of 1a-11 exhibit a $[M-1]^-$-ion, which in the cases of 3a, 3b, 6a, and 6b represents the base peak (see Tab. 3). In addition, some resonance-stabilized negative ions[21] of type $[b]^-$, $[e]^-$, $[h-2H]^-$, and $[k-H]^{-\bullet}$ are observed. The leucine, tryptophan, and glutamic acid derivatives 2, 5, and 9, respectively, show a significant ion at m/z 234 originated by loss of R^3 from the $[M-HCO_2R^1]^{-\bullet}$-ion (see Fig. 3).

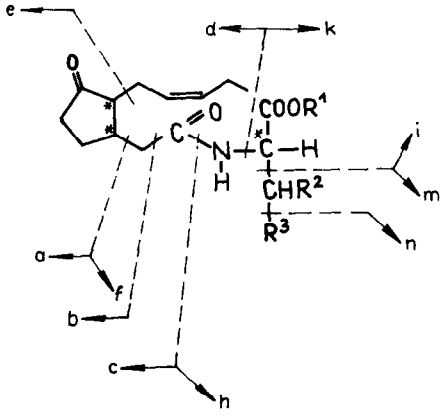


Fig. 3: General fragmentation pattern of N-(jasmonoyl)-amino acid conjugates

This ion representing a loss of isopropyl in 2a and 2b also appears in the corresponding positive ion mass spectra. It should be mentioned that an ion at m/z 234 cannot be observed in the spectra of the isoleucine conjugates 3a and 3b because of structural reasons. The negative ion mass spectra of the leucine and isoleucine compounds show remarkable differences from each other. While in 2a and 2b the resonance-stabilized ion of the type $[h-2H]^-$ at m/z 128 represents the base peak, the $[M-1]^-$-ion is the base peak of the isoleucine derivatives 3a and 3b (see Tab. 3).

Selected ^{1}H NMR data of the N-(jasmonoyl)-amino acid esters are given in Table 4.

Experimental

Materials

(±)-JA-Me was purchased from Firmenich (Geneva); isobutyl chloroformate from Merck (Darmstadt); Amberlite IRC-50 from SERVA (Heidelberg); S-valine (S-Val), S-leucine (S-Leu), S-phenylalanine (S-Phe), S-aspartic acid (S-Asp), S-glutamic acid (S-Glu), R-Val, and R-Phe from Reanal (Budapest); S-isoleucine (S-Ile), and S-tryptophan (S-Trp) from Merck; S-alanine methyl ester (S-AlaOMe), and S-tyrosine ethyl ester (S-TyrOEt) from SERVA; silica gel 60 G for TLC and silica gel 60 (0.063-0.2 mm) for CC from Merck. Tetrahydrofuran (THF) was freshly distilled; all other solvents were redistilled before use. (-)-JA has been isolated from the fungus Botryodiplodia theobromae by H.-D. Knöfel according to [21].

Methods

The following solvent systems were used for both TLC (20 x 20 cm, 0.3 mm) and column chromatography (CC):

A	n-hexane-EtOAc-HOAc	= 60 : 40 : 1	(v/v)
B	$CHCl_3$-EtOAc-HOAc	= 14 : 6 : 1	"
C	$CHCl_3$-EtOAc-HOAc	= 17 : 3 : 1	"
D	$CHCl_3$-EtOAc-MeOH-HOAc	= 12 : 3 : 5 : 1	"
E	n-hexane-EtOAc	= 2 : 3	"

For analytical detection the TLC plates were sprayed with anisaldehyde reagent and heated for 5 - 10 min at 120° C [22].

MS: The positive (10 - 16 eV) and the negative (2 - 4 eV) ion mass spectra were obtained with an electron attachment mass spectrograph built by the Research Institute 'Manfred von Ardenne', Dresden.

The ^1H NMR spectra were recorded on the Bruker spectrometer WP 200 at 200.13 MHz. The chemical shifts were measured using tetramethylsilane (TMS) as the internal standard.

IR: Specord 75 IR (VEB Carl Zeiss, Jena). The spectra were recorded in KBr discs (1a - 10) and $CHCl_3$ (11). The presence of characteristic functions was confirmed by the expected adsorptions at 3450 - 3300 cm^{-1} (OH), 1790 - 1750 cm^{-1} (ketone, ester), 1710 - 1690 cm^{-1} (acid), 1620 - 1610 cm^{-1} (amide), and 1560 - 1530 cm^{-1} (amide).

The melting points were determined on a Boetius hot-stage microscope and are corrected.

ORD: Optical rotation curves were recorded by a Jasco ORD/UV-5 spectropolarimeter in methanolic solution at room temperature.
The specific rotations were determined in methanol.

HPLC: Analytical HPLC was performed with an Hewlett Packard 1090 fitted with a 4 x 200 mm MOS (RP 8)-column (0.005 mm). Solvent : MeOH-H_2O (0.1 % H_3PO_4) = 40 : 60 (v/v), 1 ml min^{-1}, detection at 210 nm (photodiodearray).

Hydrolysis of (±)-JA-Me

500 mg (2 mmol) synthetic (±)-JA-Me dissolved in 5 ml MeOH were heated under reflux with 1 N NaOH (10 ml) for 1 h. After evaporation the aqueous phase was adjusted to pH 3 - 4 with 2 N HCl and extracted five times with 5 ml $CHCl_3$. The residue of the combined and evaporated organic phases was purified on silica gel chromatography using solvent A. The fractions were tested by TLC (systems A and B). The fractions containing the (±)-JA were collected, evaporated and dried.

Preparation of the amino acid lithium salts

Aqueous solutions of the (S)- and (R)-amino acids (4 mmol) were subjected to Amberlite IRC-50 (25 ml gel, Li^+-form). The Li-salts of the corresponding amino acid were eluted by washing with 50 ml H_2O. The eluates were lyophilized and stored at 4° C.

General procedure of the synthesis of the conjugates 1 - 9

Purified (±)-JA (210 mg, 1 mmol) was dissolved in 5 ml dry THF containing 0.15 ml (1.08 mmol) triethylamine. Under stirring at -10° C, 0.14 ml (1.07 mmol) isobutyl chloroformate were added. After 60 min the precipitate was filtered off. The filtrate was mixed with a solution of 3.5 mmol amino acid lithium salt in H_2O or aqueous THF at 4° C. After stirring for 5 h the reaction mixture was diluted with 15 ml H_2O, evaporated, and the aqueous concentrate acidified to pH 3 - 4, filtered and extracted with 10 x 10 ml $CHCl_3$. The combined organic phases were taken to dryness. The residue was submitted to silica gel chromatography (100 g, 15 x 950 mm) using the solvent systems B (conjugates 1 - 7) and D (compounds 8 and 9), respectively. The rechromatographed diastereomers 1a - 7b were crystallized from $CHCl_3$-n-hexane (JA conjugates of Val, Leu, Ile) or EtOAc-n-hexane (conjugates with Phe and Trp).

Synthesis of the conjugates 10 and 11

87.6 mg (0.417 mmol) (-)-JA were activated with 0.446 mmol isobutyl chloroformate and 0.451 mmol triethylamine in dry THF as described for (±)-JA. The filtrated solution of the mixed anhydride was added to 1.5 mmol S-amino acid alkyl ester (155 mg S-AlaOMe or 313 mg S-TyrOEt) dissolved in 4 ml THF containing 0.25 ml triethylamine and the solution stirred for 5 h at 4° C. After evaporation the crude product was loaded onto a silica gel column (8 x 900 mm) equilibrated in solvent C. The eluted fractions containing the conjugate were collected and concentrated in vacuo. The rechromatography of the obtained residue on another silica gel column (solvent E) yielded 88.9 mg 10 and 122 mg 11, respectively. The conjugate 10 was crystallized from $CHCl_3$-n-hexane.

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