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Note

Volatile Emission by [*N*-(−)-jasmonoyl]-alanylleucine from Rice Leaves (*Oryza sativa* L.)

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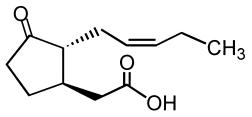
A dipeptide conjugate of jasmonic acid, [*N*-(−)-jasmonoyl]-L-alanyl -L-leucine, was prepared and its biological activities were investigated. The conjugate was active in the emission of linalool from rice leaves, while [*N*-(−)-jasmonoyl]-D-alanyl-L-leucine was not. Neither conjugate was active in the production of sakuranetin. This is the first report of a biologically active dipeptide conjugate of jasmonic acid. © Pesticide Science Society of Japan

Keywords: jasmonic acid, conjugate, alanylleucine, volatiles, rice plants.

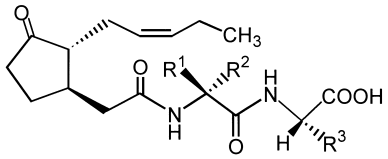
INTRODUCTION

Jasmonic acid (JA) activates plants' self-defensive systems including the induction of protease inhibitors and various secondary metabolites.[1, 2] Recent studies have shown that plants such as lima beans and corn emit volatiles as a chemical defense, and the emission is under the control of octadecanoid signaling compounds, including JA.[3–6] In rice plants, it has been shown that JA induces the emission of volatiles such as linalool and α-copaene from the leaves.[7] Although the role of the volatiles from rice leaves is still unclear, it is possible that they might activate disease resistance in neighboring leaves, or play important roles as herbivore-induced volatiles as proposed in lima beans.[3]

Derivatives of JA have been found in plants, including sugar conjugates and amino acid conjugates.[8] Tuberonic acid, a JA analogous compound with a glucose moiety, has been isolated as a tuber-inducing compound from potato.[9] Amino acid conjugates of JA are thought to work as derivatives of JA in the octadecanoid signaling pathway as well as free JA.[10] An important role for amino acid moieties in the conjugates of JA has been shown in the emission of volatiles from lima bean.[10] In rice plants, [*N*-(−)-jasmonoyl]-L-leucine and [*N*-(−)-jasmonoyl]-L-valine have been shown to elicit sakuranetin production,[11] and conjugates of JA increase in rice leaves stressed by wounding.[12] In previous studies, it was notable that these conjugates consist of JA and a single amino acid. Since the amino acid moieties of the conjugates of JA would play an important role in the activities as discussed,[10,11] we speculated that different types of conjugates of JA also might be active. Thus we designed a conjugate of JA with a dipeptide, *i.e.* a dipeptide conjugate of JA, and investigated its activity. Here, we describe the preparation of [*N*-(−)-jasmonoyl]-alanylleucine and its activity in rice leaves.



JA



[*N*-(−)-jasmonoyl]-L-alanyl-L-leucine : R^1=CH_3, R^2=H, R^3=*iso*-butyl

[*N*-(−)-jasmonoyl]-D-alanyl-L-leucine : R^1=H, R^2=CH_3, R^3=*iso*-butyl

Fig. 1. Structures of JA and *N*-jasmonoyl-alanylleucine.

MATERIALS AND METHODS

1. Chemicals

Racemic JA was prepared as described previously.[13] Successive enantiomeric separation[14] gave (−)-JA ($[\alpha]_D^{20}$=−81.6° (c=0.5, CH_3OH)), which was used for experiments. Dipeptides (L-alanyl-L-leucine and D-alanyl-L-leucine) were purchased from Sigma Chemical Co., St. Louis, Mo. USA.

2. Synthesis

Proton and ^{13}C-NMR spectra were recorded using a JEOL ECP-400 in CD_3OD with tetramethylsilane as an internal standard. Mass spectra were recorded using a PE-CIEX API-2000 Mass Spectrometer with an electro-spray ionization (ESI) source. Values of optical rotation were recorded with a JASCO Polarimeter P-1020.

2.1. Preparation of [N-(−)-jasmonoyl]-L-alanyl-L-leucine

To a THF solution (20 ml) containing (−)-JA (290 mg, 1.4 mM) and triethylamine (220 mg, 2.2 mM), isobutyl chloroformate (220 mg, 1.6 mM) was added at 0°C. After 30 min the percipitate was filtered off, and the filtrate was added to a THF solution (10 ml) containing L-alanyl-L-leucine lithium salt (1 g, 4.8 mM) and triethylamine (220 mg, 2.2 mM) at 0°C. After stirring overnight at room temperature, the mixture was concentrated.

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Water (20 ml) was added to the residue and acidified to pH 3 with HCl. The solution was extracted with chloroform (20 ml×3), and the combined organic layer was concentrated. The residue was subjected to silica gel column chromatography ($CHCl_3$: EtOAc : AcOH=14 : 6 : 1) to give an oily product. The product was crystalized from EtOAc and *n*-hexane to give [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine as a colorless powder (380 mg, 70% yield). ^{1}H-NMR δ (400 MHz in CD_3OD): 0.92 (3 H, d, J=6.3 Hz), 0.95 (3 H, t, J=7.4 Hz), 0.96 (3 H, d, J=6.6 Hz), 1.34 (3 H, d, J=7.0 Hz), 1.52 (1 H, m), 1.63 (3 H, m), 1.74 (1 H, m), 1.95 (1 H, m), 2.01–2.19 (5 H, m), 2.20–2.43 (H, m), 2.56 (1 H, dd, J=13.6, 4.4), 4.41 (2 H, m), 5.28 (1 H, m), 5.42 (1 H, m), 8.17 (1 H, brs); ^{13}C-NMR δ (100 MHz in CD_3OD): 15.3, 18.8, 22.3, 22.6, 24.2, 26.7, 27.0, 28.8, 39.4, 40.4, 42.1, 42.5, 50.9, 52.7, 56.3, 127.1, 135.4, 174.9, 175.8, 176.6, 222.6; ESI-MS (positive) *m*/*z*: 395.0 ($[M+H]^+$) and 263.9; ESI-MS (negative) *m*/*z*: 393.0 $[M-H]^-$; $[\alpha]_D^{25}=-76.7°$ (c=0.5, CH_3OH). [*N*-(−)-jasmonoyl]-D-alanyl-L-leucine was prepared in the same manner from (−)-JA and D-alanyl-L-leucine lithium salt. ^{1}H-NMR δ (400 MHz in CD_3OD): 0.92 (3 H, d, J=6.2 Hz), 0.94 (3 H, t, J=7.7 Hz), 0.95 (3 H, d, J=6.2 Hz), 1.34 (3 H, d, J=7.0 Hz), 1.53 (1 H, m), 1.60–1.74 (4 H, m), 1.96 (1 H, m), 2.01–2.19 (4 H, m), 2.20–2.43 (6 H, m), 2.61 (1 H, dd, J=13.6, 4.4), 4.43 (2 H, m), 5.28 (1 H, m), 5.41 (1 H, m), 8.08 (1 H, brs); ^{13}C-NMR δ (100 MHz in CD_3OD): 15.3, 19.3, 22.3, 22.6, 24.1, 26.8, 27.0, 28.6, 39.4, 40.4, 42.2, 42.5, 50.9, 52.8, 56.2, 127.1, 135.5, 174.8, 175.6, 176.4, 222.6; ESI-MS (positive) *m*/*z*: 395.0 ($[M+H]^+$) and 263.9; ESI-MS (negative) *m*/*z*: 393.0 $[M-H]^-$; $[\alpha]_D^{25}=-9.1°$ (c=0.5, CH_3OH).

3. *Bioassay*

The treatment of rice leaves and analysis of volatiles were performed as described as previously.[7,15] The analysis of sakuranetin in rice leaves was conducted as reported in a previous study.[16]

RESULTS AND DISCUSSION

We prepared [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine which includes L-leucine, because [*N*-(−)-jasmonoyl]-L-leucine has been found in rice leaves.[12] We also prepared [*N*-(−)-jasmonoyl]-D-alanyl-L-leucine which includes an unnatural D-alanine. The conjugates were prepared *via* mixed anhydride.[17] The structures were confirmed by NMR and mass spectrometry. In the positive mass spectrum of both conjugates, a parent ion ($[M+H]^+$, *m*/*z* 395.0) and a fragment ion (*m*/*z* 263.9) were present. The fragment ion presumably formed on loss of the leucine moiety (-NHCH($CH_2CH(CH_3)_2$)COOH=130). The treatment of rice leaves with [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine resulted in a massive amount of linalool being emitted (Table 1). The emission of linalool was strongly enhanced to 1.5 ng / sample tube (equivalent of *n*-octane as an internal standard) in the treated rice leaves after 6 hr in comparison to racemic JA as a positive control.[15] Linalool accounted for over 90% of the volatiles emitted by [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine, and its profile was the same as that by JA.[15] During the incubation with both compounds (6 hr), no phytotoxic damage including senescence was observed. In contrast, [*N*-(−)-jasmonoyl]-D-alanyl-L-leucine induced no volatile emission, suggesting that the natural L-amino acid is essential for the emission. This result is consistent with the finding that none of the conjugates containing unnatural amino acids were able to induce the production of volatiles with a JA analog in lima bean.[10]

Table 1. Effects of JA and *N*-[(−)-jasmonoyl]-alanylleucine on the emission of volatile

Compound[a]	Linalool emission[b]
water (control)	n.d.
JA (as positive control)	0.19±0.05[c]
(−)-JA-L-alanyl-L-leucine	1.50±0.19[c]
(−)-JA-D-alanyl-L-leucine	n.d.

[a] Compounds were tested as a 1 mM solution in water.

[b] ng/sample tube, equivalent of *n*-octane

[c] Data shown are from two independent experiments

Both conjugates of JA were proved to be stable against breakdown into free JA over 48 hr (1 mM solution in water at 25°C) by LC-MS analysis (data not shown), therefore free JA from the conjugate might not contribute to the emission. But it remains to be considered whether free JA yielded by enzymatic hydrolysis induces the emission, and the possibility should be verified independently in a future study. It is also possible that [*N*-(−)-jasmonoyl]-L-alanine yielded by enzymatic or nonenzymatic hydrolysis might induce the emission. [*N*-(−)-jasmonoyl]-L-alanine has been shown to be active in momilactone A production and PAL-inducing activity,[18] and it will be necessary to investigate the activity of [*N*-(−)-jasmonoyl]-L-alanine together with other conjugates of JA with a single amino acid for volatile emission activity.

Neither [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine nor [*N*-(−)-jasmonoyl]-D-alanyl-L-leucine showed elicitor activity for sakuranetin production after 48 hr treatment with 1 mM solution in water, while racemic JA showed strong activity at 100 μM as a positive control (data not shown). It is probable that [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine was active in only emission, because the system inducing the emission of volatiles was speculated to be different from that inducing production of sakuranetin.

Although the roles of free JA and its early biosynthetic precursors including 12-oxo-phytodienoic acid have been established,[19] less information has been obtained about derivatives of JA. To our knowledge, peptide conjugates of JA, including [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine, have not been isolated from natural sources, nor reported as biologically active derivatives till now. Although it is necessary to prepare other conjugates of JA, our results provide a clue to finding unknown derivatives of JA. The performance of [*N*-(−)-jasmonoyl]-L-alanyl-L-leucine prompted us to detect such conjugates in rice leaves. The identification of peptide conjugates of JA is now under investigation.

REFERENCES

1) E. E. Farmer and C. A. Ryan: *Plant Physiol*. **98**, 995–1002 (1992).
2) H. Gundlach, M. J. Müller, T. M. Kuchan and M. H. Zenk: *Proc. Natl. Acad. Sci. USA* **89**, 2389–2393 (1992).
3) T. Turlings, J. Tumlinson and W. Lewis: *Science* **250**, 1251–1253 (1990).
4) J. Hopke, J. Donath, S. Blecher and W. Boland: *FEBS Lett*. **352**, 146–150 (1994).
5) W. Boland, J. Hopke, J. Donath, J. Nüske and F. Bublitz: *Angew. Chem., Int. Ed.* **34**, 1600–1602 (1995).
6) T. Turlings, J. Loughrin, P. McCall, U. Röse, W. Lewis and J. Tumlinson: *Proc. Acad. Sci. USA* **92**, 4169–4174 (1995).
7) N. Obara, M. Hasegawa and O. Kodama: *Biosci. Biotechnol. Biochem*. **66**, 2549–2559 (2002).
8) M. Hamberg and H. Gardner: *Biochim. Biophys. Acta* **1165**, 1–18 (1992).
9) T. Yoshihara, E. Omer, H. Koshino, S. Sakamura, Y. Kikuta and Y. Koda: *Agric. Biol. Chem*. **53**, 2835–2837 (1989).
10) T. Kurumm, K. Bandemer and W. Boland: *FEBS Lett*. **377**, 523–529 (1995).
11) S. Tamogami, R. Randeep and O. Kodama: *FEBS Lett*. **401**, 239–242 (1997).
12) S. Tamogami and O. Kodama: *J. Chromatogr. A* **822**, 310–315 (1998).
13) G. Büchi and B. Egger: *J. Org. Chem*. **36**, 2021–2023 (1971).
14) R. Kramell, G. Schneider, J. Schmid, G. Sembdner and K. Schreiber: *Z. Naturforsh. B: Chem. Sci*. **45**, 377–381 (1990).
15) M. Suzuki, M. Hasegawa, O. Kodama and H. Toshima: *Biosci. Biotechnol. Biochem*. **68**, 1617–1620 (2004).
16) R. Rakwal, S. Tamogami and O. Kodama: *Biosci. Biotechnol. Biochem*. **60**, 1046–1048 (1996).
17) R. Kramell, J. Schmid, G. Schneider, G. Sembdner and K. Schreiber: *Tetrahedron* **44**, 5791–5807 (1988).
18) Y. Jikumaru, T. Asami, H. Seto, S. Yoshida, T. Yokoyama, N. Obara, M. Hasegawa, O. Kodama, M. Nishiyama, K. Okada, H. Nojiri and H. Yamane: *Biosci. Biotechnol. Biochem*. **68**, 1461–1466 (2004).
19) H. Dittrich, T. Kutchan and M. Zenk: *FEBS Lett*. **1**, 33–36 (1992).