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Leucine and Isoleucine Conjugates of 1-Oxo-2,3-dihydro-indene-4-carboxylic Acid: Mimics of Jasmonate Type Signals and the Phytotoxin Coronatine**

Thomas Krumm and Wilhelm Boland*

Institut für Organische Chemie und Biochemie, Gerhard-Domagk-Strasse 1, D-53121 Bonn, Germany. Fax +49-228735388 (Boland@uni.bonn.de)

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Abstract

Leucine and isoleucine conjugates of 1-oxo-2,3-dihydro-indene-4-carboxylic acid (1-oxo-ICA) such as **2a-e** are powerful elicitors of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*). The compounds are structurally and, with a few exceptions, functionally related to coronatin **1**, a phytotoxin from pathovars of *Pseudomonas spp.* Coronatin **1** and the conjugates of 1-oxo-ICA **2a-e** seem to mimic signals from the octadecanoid signalling pathway, like, for example, amino acid conjugates of jasmonic acid.

Keywords: Coronatine, jasmonic acid, 1-Oxo-indanoyl-isoleucine, volatile induction, amino acid conjugates, elicitors

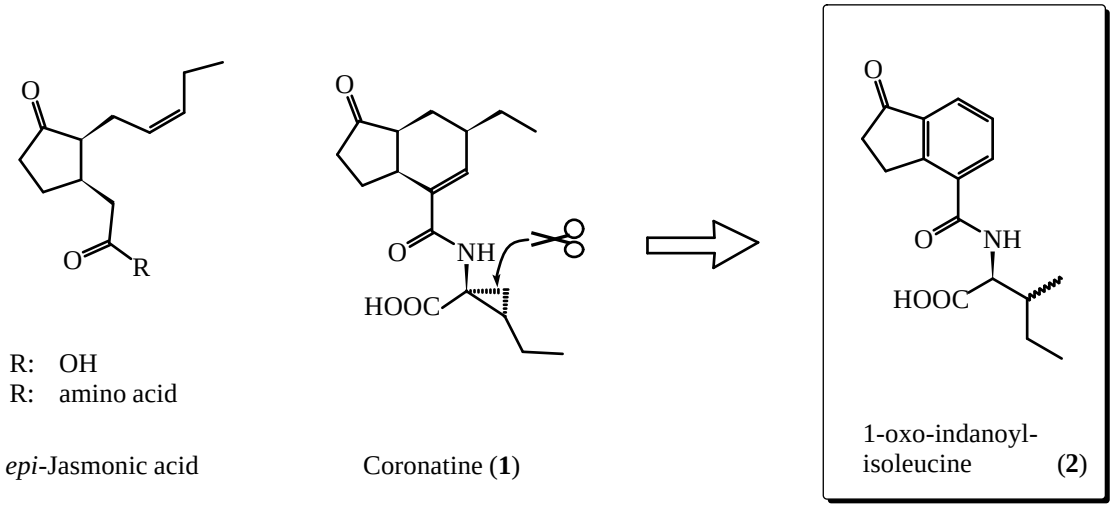
Introduction

Coronatine **1** is a powerful phytotoxin which was first isolated by *Ichihara et al*. from the fermentation broth of certain *Pseudomonas syringae* pathovars.[1] Recent studies have established that **1** exhibits a range of biological activities resembling those of jasmonic acid (JA). Common activities include induction of potato tuberisation, [2] expansion of potato cells,[3] inhibition of the growth of soybean callus,[4] induction of ethylene production in tobacco leaves,[5] induction of coiling of the touch-sensitive tendrils of *Bryonia dioica*,[6] senescence promotion in oat leaves,[7] and induction of the biosynthesis of volatiles in many plants,[8] (for reviews see ref. 9,10).

Coronatine **1** is active in all of the above examples, but its activity is generally 100 – 10000 times higher than that of JA in terms of the threshold concentration for activity.[8,11] Taking into account that amide bonds with α-branched or conformationally strained cyclic amino acids are often resistant towards hydrolysis, one could consider coronatine **1** as a structural analogue of an amino acid conjugate of *epi*-jasmonic acid. Thus, formal cleavage of the C(2)-C(3) bond of the cyclopropyl amino acid transforms this building block into isoleucine; corresponding amides with coronafacic acid [12] or JA [13] have been described. Further simplification of **1** leads to 1-oxo-ICA **3** and its conjugates with the amino acids isoleucine and leucine **2a-e** which are the first members of a novel class of artificial plant hormones. 1-Oxo-ICA **3** is readily available in larger quantities following the protocol of *Aono et al.* [14] High yields of amides (> 78%) are obtained using an amino acid ester (methyl or allyl), dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) for activation of the 1-oxo-ICA.[15] The procedure (Scheme 2) is applicable to JA as well as 1-oxo-ICA **3** and gives consistently higher yields with various amino acids than the method previously reported for the preparation of conjugates of jasmonate.[16]

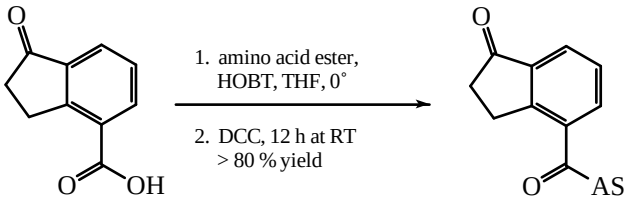
The biological activity of the conjugates **2a-e** is demonstrated by their ability to induce the biosynthesis of volatiles in 14-day-old cotyledons of the Lima bean (*Phaseolus lunatus*).[17] Conjugates of the natural *L*-amino acids Leu

* *To whom correspondence should be addressed*



epi-Jasmonic acid Coronatine (**1**)

Scheme 1



3 **2a-e**

Scheme 2

and Ile proved to be highly active (standard assay conditions: test substance at 1 µmol·ml^{-1}; however, the threshold concentrations are significantly lower, 20 nmol·ml^{-1} for **2a**), while conjugates with *D*-amino acids yielded inactive products only (cf. Table 1). Preliminary results indicate that the conjugates exhibit a slightly different induction profile than coronatine **1** or JA, respectively.[17]

Table 1

Amino acid (AS)	No.	Yield (%) (isolated)	Induction of volatiles
L-Ile	**2a**	84	active
D-Ile	**2b**	87	inactive
L-allo-Ile	**2c**	78	weakly active
L-Leu	**2d**	89	active
D-Leu	**2e**	87	inactive

The major advantage of the amino acid conjugates of 1-oxo-ICA is the *complete lack of bioactivity of their building blocks*, namely the amino acid and 1-oxo-ICA. Therefore, activity can be confidently attributed to the conjugates, rather than their hydrolysis products. Thus, conjugates of the type **2a-e** represent exceedingly valuable tools for unraveling the biological significance of amino acid conjugates of JA and coronatine **1**. Induction of volatiles can also be provoked by application of jasmonoyl (iso)leucine to leaves of the Lima bean (unpublished), but the results need to be independently verified, since an enzymatic hydrolysis of the amide bond yields JA which in turn may act as a signal. Consistent with the high activity of the conjugates, derivatives of JA which do not allow the formation of an amide bond turned out to be inactive (unpublished). Whether, and to what extent, other coronatine **1** or jasmonate inducible events can also be provoked by the novel conjugates **2a-e**, and related derivatives, is unknown and warrants for future research. First results on the induction of low molecular phytoalexins in other plants are encouraging and will be reported in due course.

Experimental Part

General. Reactions were performed under Ar. Solvents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying. Melting points are not corrected. ^{1}H- and ^{13}C NMR: *Bruker AM400* and *Bruker WM 400*; $CDCl_3$. IR: *Perkin-Elmer-1605* FT-IR spectrophotometer. MS: *Fisons MD 800* GLC/MS system and *Finnigan ITD 800* combined with a *Carlo-Erba* gas chromatograph, model *Vega*, equipped with a fused-silica capillary *SE 30* (15m × 0.32 mm); carrier gas, He at 30cm/s; scan range: 35-350 Dalton/s. Analytical GLC:

Carlo-Erba gas chromatograph, *HRGC 5300, Mega* series, equipped with fused silica capillaries, *SE 30* (10m × 0.32mm); H_2 at 30 cm/s as carrier. Silica gel, *Si 60*, (0.040-0.063 mm, *Merck*, Darmstadt, Germany) was used for liquid column chromatography

Amino acid conjugates of 1-oxo-indan-4-carboxylic acid (**2a-e**). *General procedure*

A well stirred solution of 1-oxo-indan-4-carboxylic acid **3** (1 mmol), the amino acid methylester hydrochloride (1 mmol), 4-ethylmorpholine (1 mmol), and 1-hydroxybenzotriazole (HOBT) (ca. 1.5 mmol) in THF (10 ml) is cooled to 0°, and *N,N'*-dicyclohexylcarbodiimide (1,1 mmol) is added. Stirring is continued at RT for 20 h. The precipitated urea is removed, and the solvent is replaced by ethyl acetate (10 ml). The ethyl acetate solution is washed with 2N HCl (10 ml) and with sat. aq. $NaHCO_3$ (10 ml), dried and concentrated. The amino acid conjugates are readily purified by chromatography on silica gel using either ether/hexane (2:1, v:v) or ethyl acetate/hexane (1:1, v:v) for elution.

1-Oxo-indanoyl-L-*isoleucine methylester* (**2a**)

Colourless solid. Yield: 84%. M.p.: 109°. IR (KBr): 3303, 2971, 2876, 1738, 1717, 1634, 1578, 1534, 1470, 1427, 1354, 1333, 1266, 1234, 1200, 1182, 1150, 1076, 1046, 982, 925, 822, 803, 786, 766, 738, 658. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.88-0.95 (*m*, 6 H); 1.14-1.27 (*m*, 1 H); 1.40-1.52 (*m*, 1 H); 1.92-2.03 (*m*, 1 H); 2.64 (*t*, *J* = 6.0 Hz, 2 H); 3.27-3.42 (*m*, 2 H); 3.72 (*s*, 3 H); 4.76 (*dd*, *J* = 8.5, 4.8 Hz, 1 H); 6.57 (*d*, *J* = 8.5 Hz, 1 H); 7.39 (*t*, *J* = 7.4 Hz, 1 H); 7.80 (*d*, *J* = 7.4 Hz, 2 H). ^{13}C-NMR ($CDCl_3$): 11.7; 15.6; 25.4; 26.1; 36.1; 38.2; 52.3; 56.8; 126.5; 127.7; 132.7; 133.0; 138.3; 154.0; 166.7; 172.5; 206.4. MS (EI, 70 eV): 303 (M^+, 2), 247 (4), 244 (13), 176 (15), 159 (100), 131 (23), 103 (32), 77 (15). HR-MS: 303.1479 ($C_{17}H_{21}NO_4^+$, M^+, calc. 303.1471). Anal. calc. for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 66.81; H, 7.01; N, 4.43.

1-Oxo-indanoyl-D-*isoleucine methylester* (**2b**)

Colourless solid. Yield: 87%. Analytical data identical with 1-oxo-indanoyl-*L*-isoleucine methylester (**2a**). HR-MS: 303.1480 ($C_{17}H_{21}NO_4^+$, M^+, calc. 303.1471).

1-Oxo-indanoyl-L-*alloisoleucine methylester* (**2c**)

Colourless solid. Yield: 78%. M.p.: 80°. IR (KBr): 3278, 3052, 2967, 2876, 1743, 1717, 1630, 1580, 1535, 1471, 1434, 1331, 1263, 1236, 1203, 1159, 1044, 984, 924, 802, 765, 667. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, *J* = 7.0 Hz, 3 H); 0.94 (*t*, *J* = 7.0 Hz, 3 H); 1.14-1.26 (*m*, 1 H); 1.41-1.52 (*m*, 1 H); 1.96- 2.06 (*m*, 1 H); 2.67 (*t*, *J* = 6.0 Hz, 2 H); 3.28-3.44 (*m*, 2 H); 3.73 (*s*, 3 H); 4.88 (*dd*, *J* = 8.9, 4.0 Hz, 1 H); 6.41 (*d*, *J* = 8.9 Hz, 1 H); 7.41 (*t*, *J* = 7.6 Hz, 1 H); 7.81 (*d*, *J* = 7.6 Hz, 1 H); 7.84 (*d*, *J* = 7.6 Hz, 1 H). ^{13}C-NMR ($CDCl_3$): 11.8; 14.8; 26.0; 26.4; 36.1; 37.8; 52.4; 55.7; 126.3; 127.6; 132.8; 133.2; 138.1; 154.0; 167.1; 172.8; 206.4. MS (EI, 70 eV): 303 (M^+, 2), 247 (12), 244 (17), 176 (10), 159 (100), 131 (20), 103 (24), 77 (14). HR-MS: 303,1468 ($C_{17}H_{21}NO_4^+$, M^+, calc. 303.1471).

1-Oxo-indanoyl-L-*leucine methylester* (**2d**)

Colourless solid. Yield: 89%. M.p.: 89°. IR (KBr): 3246, 3052, 2955, 1752, 1720, 1648, 1624, 1581, 1541, 1436, 1362, 1332, 1268, 1232, 1199, 1162, 1045, 983, 919, 829, 800, 764, 679. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.92 (*d*, *J* = 6.2 Hz, 3 H); 0.94 (*d*, *J* = 6.2 Hz, 3 H); 1.57-1.75 (*m*, 3 H); 2.62 (*t*, *J* = 6.0 Hz, 2 H); 3.25-3.40 (*m*, 2 H); 3.71 (*s*, 3 H); 4.75-4.82 (*m*, 1 H); 6.53 (*d*, *J* = 8.1 Hz, 1 H); 7.36 (*t*, *J* = 7.6 Hz, 1 H); 7.78 (*d*, *J* = 7.6 Hz, 2 H). ^{13}C-NMR ($CDCl_3$): 22.0; 22.8; 25.1; 26.0; 36.1; 41.7; 51.1; 52.5; 126.4; 127.6; 132.6; 132.8; 138.3; 154.3; 166.8; 173.6; 206.5. MS (EI, 70 eV): 303 (M^+, 1), 247 (12), 244 (8), 159 (100), 144 (4), 131 (20), 103 (22), 77 (11). HR-MS: 303.1470 ($C_{17}H_{21}NO_4^+$, M^+, calc. 303.1471).

1-Oxo-indanoyl-D-*leucine methylester* (**2e**)

Colourless solid.. Yield: 87%. Analytical data identical with 1-oxo-indanoyl-*L*-leucine methylester (**2d**). HR-MS: 303.1473 ($C_{17}H_{21}NO_4^+$, M^+, calc. 303.1471).

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References and Notes

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