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Strategy for synthesis of the isoleucine conjugate of *epi*-jasmonic acid

Narihito Ogawa, Yuichi Kobayashi *

Department of Biomolecular Engineering, Tokyo Institute of Technology, B52, Nagatsuta-cho 4259, Midori-ku, Yokohama 226-8501, Japan

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ABSTRACT

The TES ether of 2-((1*R*,2*S*,3*R*)-3-hydroxy-2-((*Z*)-pent-2-enyl)cyclopentyl)acetic acid (**5**, equal to the reduction product of *epi*-jasmonic acid) derived from (1*R*,4*S*)-4-hydroxycyclopent-2-enyl acetate (**19**) in 13 steps was activated by using isobutyl chloroformate and was subjected to condensation with isoleucine at room temperature for 48 h. The product was desilylated and oxidized to the isoleucine conjugate of *epi*-jasmonic acid in 68% yield over three steps. Similarly, allo-isoleucine conjugate of *epi*-jasmonic acid and three isoleucine conjugates of *ent-epi*-jasmonic acid, jasmonic acid, and *ent*-jasmonic acid were synthesized.

Jasmonates regulate stress responses and development in plants.[1,2] Recently, the jasmonate ZIM-domain 1 (JAZ1) protein was elucidated as the key component of the jasmonate signaling.[3] This protein acts to repress transcription of jasmonate-responsive genes and is degraded through the SCF^{COI1} ubiquitin ligase-dependent 26S proteasome pathway. Isoleucine conjugate of 'jasmonic acid'[4] assists the binding of SCF^{COI1} to JAZ1 and the subsequent degradation. In connection with these findings, structure–activity relationship of the amino acid part was briefly examined to find the high activity for the isoleucine conjugate. On the other hand, structural requirement of the 'jasmonic acid' part appears ambiguous because of the use of stereoisomeric mixtures.

The metabolism of linolenic acid has been studied actively to disclose the functions in plants.[1] One important discovery is the enzyme-promoted production of 12-oxo-PDA (**1**) with the 9*S*,13*S* chirality (i.e., cis configuration for the two side chains), which undergoes reduction and subsequent β-oxidations[5] (three times) to produce *epi*-jasmonic acid (**2**) (Fig. 1). During the transformation, the cis configuration is strictly conserved, though the chiral center next to the carbonyl group is susceptible to epimerization to the thermodynamically more stable trans isomers. An equilibrium ratio of the methyl ester of **2** (i.e., methyl *epi*-jasmonate) and the *trans* isomer (methyl jasmonate) at ambient temperatures is 5:95,[6] which is supported by calculation based on the molecular mechanics.[7] Similar equilibrium ratios are suspected for the other metabolites. In contrast to the thermodynamic instability, however, 12-oxo-PDA (**1**) and tuberonic acid (**4**) were proven kinetically quite stable under neutral conditions.[8,9] Consequently, isolation and/or purification appear to involve step(s) that is responsible for the contamination of the trans isomers.

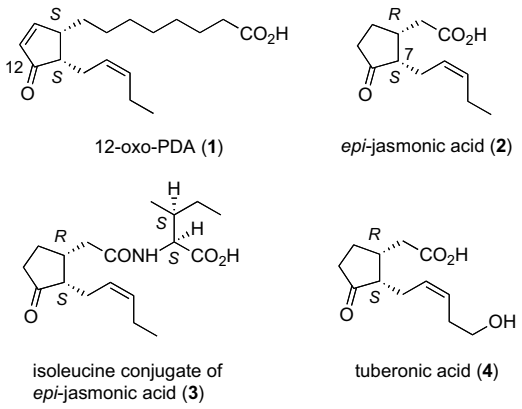

Figure 1. Some of naturally occurring metabolites of linolenic acid.

Regarding the acid part of the isoleucine conjugate, the higher content of *epi*-jasmonic acid (32%) than that at equilibrium (5% by analogy with methyl *epi*-jasmonate) is determined by GC–MS for the samples, which were carefully prepared taking account of the possibility of the epimerization.[10a,b] The ratio is suggestive of urgent biological investigation using the isoleucine conjugate of *epi*-jasmonic acid, that is, **3**.

Previously, condensation of jasmonic acid (trans isomer) with amino acids had been reported.[11] However, no yields are given, and direct application to *epi*-jasmonic acid seems difficult due to possibility of the epimerization.[12] As a consequence, we envisioned a method delineated in Scheme 1 as a secure approach to **3**. Epimerization-free acid **5** was selected as an *epi*-jasmonic acid precursor. The acid **5** was prepared as shown in Scheme 1 starting with **9**, which was an intermediate for the synthesis of tuberonic acid (**4**).[9] Herein, we report results of this investigation and

* Corresponding author. Tel./fax: +81 45 924 5789.
E-mail address: ykobayas@bio.titech.ac.jp (Y. Kobayashi).

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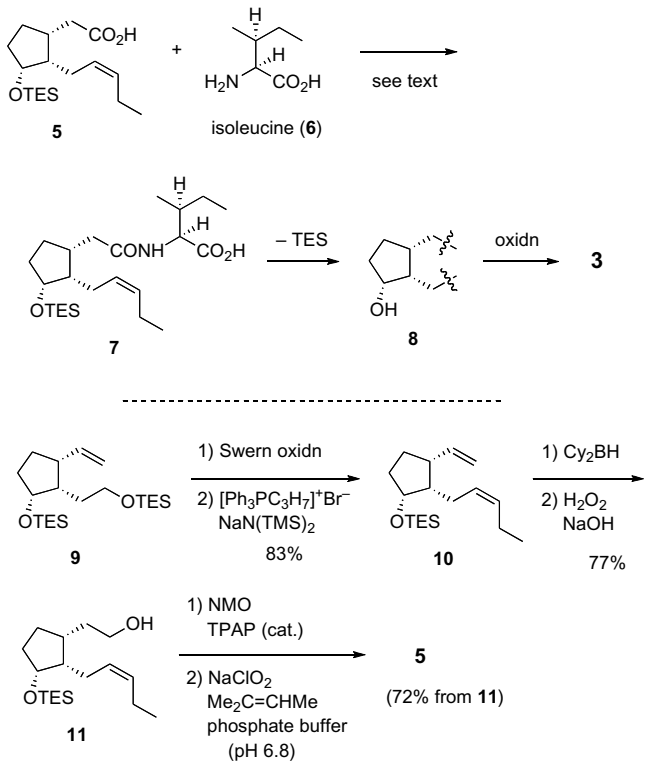


Scheme 1. An approach to the conjugate **3**.

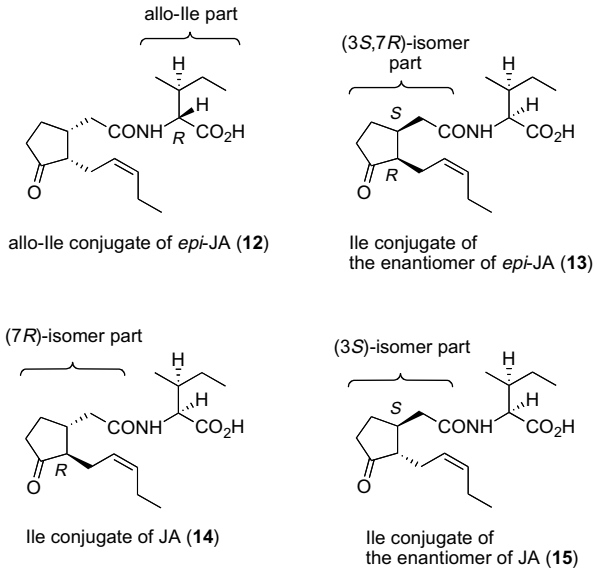
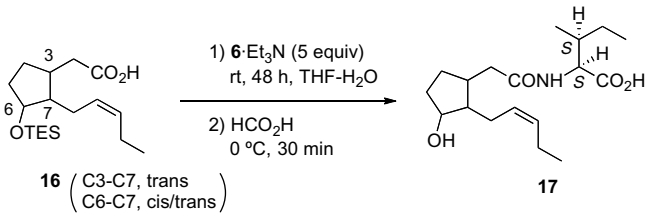


Figure 2. Related compounds of the isoleucine conjugate.

application to synthesis of isomers **12**–**15** (Fig. 2). Stability of **3** is also presented.

For preliminary study of the condensation, a diastereomeric mixture of **16** was prepared in good yield from racemic methyl jasmonate by a sequence of reactions: (1) NaOH, (2) $NaBH_4$, (3) TESCl, imidazole. The acid **16** was activated by reagents given in Table 1 and was subjected to condensation with isoleucine (**6**). In brief, **16** and the reagent were mixed at room temperature for 3 h and the condensation with the isoleucine and Et_3N complex **6**·Et_3N (5 equiv) was examined at room temperature for 48 h.

Table 1
Condensation of a model acid **16**[a] with **6**[b]



Entry	Reagent[c] (equiv)	Yield[d] (%)
1	WSC (1.3)	43
2	DMT-MM (1.3)	51
3	BOPCl (1.3)	47
4	HATU (1.3)	47
5	HBTU (1.3)	51
6	TCBC (1.5)	17
7	DCBC (1.5)	0
8	Isobutyl chloroformate (1.3)	60
9	1-Chloroethyl chloroformate (1.3)	42

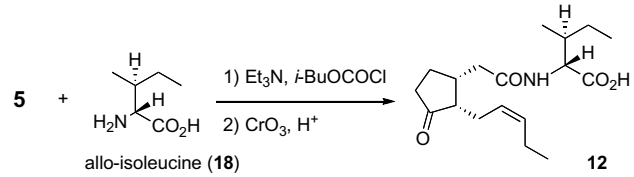
[a] A diastereomeric mixture from racemic methyl jasmonate.
[b] Condensations with **6**·Et_3N (5 equiv) were carried out in THF and H_2O (1:1) at room temperature for 48 h.
[c] WSC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; DMT-MM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; BOPCl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo(4,5-*b*) pyridinium 3-oxide hexafluorophosphate; HBTU, 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium 3-oxide hexafluorophosphate; TCBC, 2,4,6-trichlorobenzoyl chloride; DCBC, 2,6-dichlorobenzoyl chloride.
[d] Isolated yields by chromatography on silica gel.

The TES group in the product was removed in HCO_2H and the resulting alcohol **17** was purified by chromatography on silica gel ($CHCl_3$/EtOAc to $CHCl_3$/EtOAc/HCO_2H). Among the reagents, isobutyl chloroformate gave a good yield (entry 8). In addition, further condensation product(s) such as **16**-Ile–Ile was not identified.

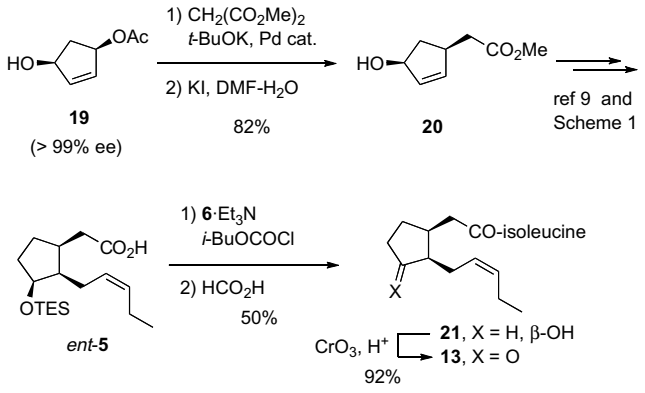
The above procedure was applied to the optically active acid **5** successfully, and removal of the TES group in HCO_2H produced cyclopentanol **8** in 68% yield. Finally, Jones oxidation (CrO_3, H_2SO_4) at 0 °C for 30 min gave the isoleucine conjugate **3** quantitatively.[13] The isomeric purity of **3** over the trans isomer (**14** in Fig. 2) was 96% by 500 MHz ^{1}H NMR spectroscopy.[14a] The acidic conditions during the oxidation are responsible for the partial (4%) epimerization to **14**. We repeated the procedure several times with similar ratios. The conjugate **3** with this purity will be acceptable for biological study.

Next, we turned our attention to synthesis of the stereoisomers **12**–**15**. Condensation of acid **5** with allo-isoleucine **18** (purchased from Aldrich) followed by desilylation and subsequent Jones oxidation furnished **12** in 48% with a similar efficiency (Scheme 2). The epimeric purity at the C7 carbon (next to the carbonyl carbon) was 94% by ^{1}H NMR spectroscopy.

Synthesis of **13**, an isoleucine conjugate with the enantiomer of *epi*-jasmonic acid, started with **19** (>99% ee by chiral HPLC). As delineated in Scheme 3, **19** (>99% ee) was converted to **20** by palladium-catalyzed reaction with methyl malonate followed by



Scheme 2. Synthesis of allo-Ile conjugate of *epi*-JA (**12**).

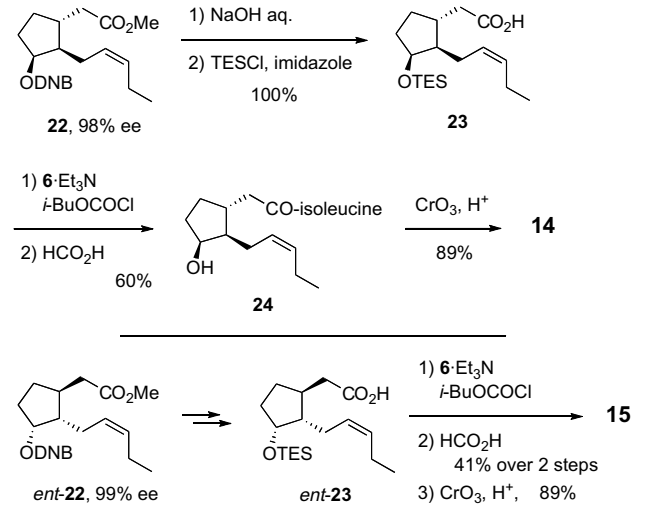


Scheme 3. Synthesis of Ile conjugate of the enantiomer of *epi*-JA (**13**).

decarboxylation in 82% yield.[15] Transformations of **20** to *ent*-**9** (structure not shown) and to *ent*-**5** from *ent*-**9** were accomplished similarly.[9] Finally, condensation with isoleucine (**6**) under the conditions established above followed by desilylation gave **21** in 50% yield and subsequent oxidation furnished **13** in 92% yield. The diastereomeric ratio of **13** at C7 was 96:4 by ^{1}H NMR spectroscopy.

For synthesis of isoleucine conjugates **14** and **15**, optically active intermediates **22** and the enantiomer *ent*-**22** were obtained by the kinetic resolution of the racemic alcohol derived from methyl jasmonate by PPL-assisted acetylation according to the literature procedure.[16] As summarized in Scheme 4, **22** was treated with aqueous NaOH, and the hydroxy acid was converted to the TES-protected acid **23**. Condensation with **6**·Et$_3$N followed by deprotection afforded **24**, which was oxidized to **14** in 89% yield. Diastereomeric purity of **14** over **3** was >99% by 500 MHz. ^{1}H NMR spectroscopy, indicating slower (if any) deprotonation of **14** at C7 than that of **3** (4%) during the oxidation. The stereochemistry of the C3 acetamide chain on the cyclopentanone ring might be responsible for the difference between the two cases. In a similar manner, *ent*-**22** was transformed to **15** without detectable epimerization by ^{1}H NMR spectroscopy.

With the ^{1}H NMR spectra of **3** and its C7 epimer **14** in hand, we examined chemical stability of **3** in methanol by ^{1}H NMR spectroscopy.[14b] In contrast to the expectation from the partial epimerization observed during Jones oxidation, conjugate **3** was proven to be



Scheme 4. Synthesis of isoleucine conjugates of jasmonic acid and enantiomer of jasmonic acid. DNB = 3,5-$(NO_2)_2C_6H_3C(=O)$–.

quite stable at room temperature for three weeks, whereas addition of K_2CO_3 promoted rapid epimerization to afford a 6:94 mixture of **3** and **14** after 24 h. The rate of the epimerization appears faster than that of tuberonic acid (**4**) and much faster than 12-oxo-PDA (**1**), though we do not have any reason to explain such a difference.

In summary, we developed an access to **3** for the first time, and the method was successfully applied to synthesis of its epimers **12**–**15**. These compounds will be useful not only for the biological study at molecular level but also as standards for elucidation of these compounds from natural sources.

Acknowledgments

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12. An attempted condensation of *epi*-jasmonic acid (**2**) and isoleucine (**6**) under the optimized conditions (Table 1, entry 8) gave a mixture of the desired product **3** and trans isomer **14** in a 30:70 ratio.
13. To a solution of acid **5** (25.0 mg, 0.0766 mmol) in THF (1 mL) were added Et$_3$N (0.014 mL, 0.099 mmol) and isobutyl chloroformate (0.013 mL, 0.10 mmol). The mixture was stirred at room temperature for 3 h. The mixture was filtered off, and the precipitates were washed with THF (3 mL). A solution of isoleucine (**6**) (50 mg, 0.38 mmol) and Et$_3$N (0.053 mL, 0.38 mmol) in H_2O (4 mL) were added to the combined filtrates. The mixture was stirred at room temperature for 48 h, and diluted with saturated NH_4Cl. The resulting mixture was extracted with $CHCl_3$ several times. The combined extracts were dried over $MgSO_4$ and concentrated. The residue was diluted with HCO_2H (2 mL) at 0 °C. After 30 min, the solution was concentrated to give a residue, which was purified by chromatography on silica gel ($CHCl_3$/EtOAc to $CHCl_3$/EtOAc/HCO_2H) to give alcohol **8** (17.0 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 0.95 (d, J = 7.5 Hz, 3H), 0.97 (t, J = 7.5 Hz, 3H), 1.11–1.71 (m, 5H), 1.82–2.31 (m, 9H), 2.55 (dd, J = 13.8, 3.9 Hz, 1H), 4.23 (t, J = 4.8 Hz, 1H), 4.61 (dd, J = 8.4, 4.8 Hz, 1H), 4.0–4.9 (br s, 2H), 5.33–5.50 (m, 2H), 6.10 (d, J = 8.4 Hz, 1H). To a solution of the above alcohol **8** (7.9 mg, 0.024 mmol) in acetone (1 mL) was added Jones reagent (2 drops, 4 M solution) at 0 °C. The resulting mixture was

stirred at 0 °C for 30 min, and *i*-PrOH was added to quench excess reagent. The mixture was subjected directly to chromatography on silica gel ($CHCl_3$/EtOAc/HCO_2H) to give the isoleucine conjugate of *epi*-jasmonic acid **3** (7.9 mg, 100%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, J = 7.5 Hz, 3H), 0.95 (d, J = 7.5 Hz, 3H), 0.96 (t, J = 7.5 Hz, 3H), 1.16–1.38 (m, 2H), 1.44–1.54 (m, 1H), 1.82–2.10 (m, 7H), 2.19–2.30 (m, 2H), 2.34–2.44 (m, 2H), 2.84–2.94 (m, 1H), 4.63 (dd, J = 8.5, 4.5 Hz, 1H), 5.34 (dt, J = 10.5, 7.5 Hz, 1H), 5.45 (dt, J = 10.5, 7.5 Hz, 1H), 6.30 (d, J = 8.5 Hz, 1H), 6.1–6.8 (br s, 1 H).

14. (a) Compared the signals at d 2.84–2.94 (m) and 2.64–2.71 (dd, J = 14 and 7 Hz) for **3** and the trans isomer (**14**) (500 MHz, $CDCl_3$).; (b) Compared the signals at δ 2.76–2.90 (m) and 2.57–2.66 (m) for **3** and the isomer (**14**) (500 MHz, methanol-d_4).
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