Stereo-Selective Preparation of Teneraic Acid, trans-(2S,6S)-Piperidine-2,6-dicarboxylic Acid, via Anodic Oxidation and Cobalt-Catalyzed Carbonylation

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Teneraic acid (piperidine-2,6-dicarboxylic acid) is a naturally occurring imino acid that comprises three stereoisomers due to its two asymmetric centers at C2 and C6. The configuration of natural teneraic acid is reported to correspond to trans-(2S,6S)-teneraic acid. However, a few studies are focused on the stereospecific synthesis of trans-(2S,6S)-teneraic acid. The present study investigates a convenient synthetic method that includes regiospecific anodic oxidation and stereospecific cobalt-catalyzed carbonylation to obtain trans-(2S,6S)-teneraic acid. Methyl (S)-N-benzoyl-α-methoxypippecolate, the key intermediate that displays a structure that corresponds to an intermediate (Nα-hydroxyalkyl amide) of intramolecular amidocarbonylation, was obtained via an anodic oxidation of methyl (S)-N-benzoylpippecolate. Subsequently, cobalt-catalyzed carbonylation converted the methyl (S)-N-benzoyl-α-methoxypippecolate to trans-(2S,6S)-N-benzoylteneraic acid dimethyl ester in good optical purity (>95% enantiomeric excess (ee)) and modest yield (63%). Finally, de-protection occurred via acidic hydrolysis to obtain trans-(2S,6S)-teneraic acid. The stereochemistry of synthesized teneraic acid was confirmed as corresponding to trans-(2S,6S) by comparing its physical properties with those of a cis-meso-isomer and those of a trans-(2S,6S)-isomer that were reported in previous studies.

Key words teneraic acid; piperidine-2,6-dicarboxylic acid; stereoisomer; amidocarbonylation; anodic oxidation; carbonylation

Teneraic acid (also termed as piperidine-2,6-dicarboxylic acid) is a naturally occurring imino acid derivative that was isolated from a red alga, Porphyra tenera, which comprises three stereoisomers, namely, trans-(2S,6S)-, trans-(2R,6R)-, and cis-meso-isomers due to two asymmetric centers at C2 and C6 (Fig. 1). A study by Kawauchi et al. reported that the stereochemistry of natural teneraic acid corresponded to trans.3) Additionally, cis–meso-isomers of teneraic acid derivatives are readily obtained by platinum(IV) oxide (PtO₂) or palladium on carbon (Pd/C) catalyzed hydrogenation of pyridine-2,6-dicarboxylic acid derivatives, respectively.2)–4) Mitsakos et al. reported on dihydrodipippecolinate synthase inhibition activity as a biological activity of teneraic acid in vivo.3) However, stereoselective total synthesis of an optically active trans-isomer was not achieved until a study by Wasmann et al. who synthesized a trans-(2S,6S)-isomer by multistep reactions and determined the absolute stereochemistry of natural teneraic acid as corresponding to trans-(2S,6S).5) Ohtani et al. obtained optically pure trans-(2R,6R)- or trans-(2S,6S)-isomers via a photocatalytic reaction with activated cadmium sulfide (CdS) particles by using (R,R)- or (S,S)-2,6-diaminopimelic acid as the starting material wherein the mechanism resembles a possible biosynthetic pathway.7)–9) However, optically active 2,6-diaminopimelic acid is expensive. The following two procedures have been reported for synthesizing a racemate. Chryystal et al. synthesized racemic teneraic acid derivative via a,d’-dibromination of pimelic acid followed by cyclization using liquid ammonia.2) Einhorn et al. synthesized racemic N-tert-butoxycarbonylteneraic acid dimethyl ester via alkylation of the dianion of N-tert-butoxycarbonylteneraic acid dimethyl ester with diiodopropane.10) Additionally, Chryystal et al. separated cis–meso-isomer and racemic trans-isomer by using fractional precipitation of racemic mixture of teneraic acid diamide.11) However, it is essential to develop additional facile and practical synthesis methods with respect to trans-teneraic acid.

Conversely, cobalt-catalyzed amidocarbonylation was developed by Wakamatsu et al. in our laboratories as a useful method for the synthesis of N-acetyl-α-amino acids (6) from amide (4), aldehyde (5), and carbon monoxide (CO)11–13) (Chart 1). Dichalcogenocarbonyl [Coₓ(CO)ₙ] functions as the catalyst precursor. The first step of the amidocarbonylation involves a nucleophilic addition of the amide (4) to the aldehyde (5) with the formation of an Nα-hydroxyalkyl amide (7), which eliminates the hydroxide ion (OH⁻) to form the corresponding N-acylimine cation (8a) or N-acyliminium ion (8b). An active catalyst species, tetracarbonylcobaltate [Co(CO)₅]⁻, forms a corresponding alkyl cobalt complex (9) with the N-acylimine...
cation (8a) in a nucleophilic substitution. CO insertion transforms alkyl cobalt complex (9) into an acyl cobalt complex (10). This product (10) forms N-acyl-amino acid (6) via an intermolecular nucleophilic attack of water on the acyl bond. The intramolecular formation of an oxazolone derivative (11) with subsequent hydrolysis also seems possible. In addition to original cobalt-catalyzed amidocarbonylation, Beller developed palladium-catalyzed amidocarbonylation for the synthesis of numerous N-acyl-α-amino acid derivatives. The scope of application range of this reaction is significantly broadened with its combination with other reactions to yield intermediates that are similar to intermediates 7 or 8 in Chart 1. The synthetic application of cobalt-catalyzed amidocarbonylation to synthesize functionalized N-acyl-α-amino acid derivatives was extended by establishing stereo-selective synthesis of 6-substituted piperolic acid via an anodic oxidation–cobalt-catalyzed carbonylation reaction.

The present study provides a facile stereo-specific route to obtain trans-(2S,6S)-teneraic acid from (S)-piperolic acid by using regioselective anodic oxidation and stereospecific cobalt-catalyzed carbonylation. Furthermore, the details of characterization of trans-(2S,6S)-teneraic acid are presented in the study.

Results and Discussion

Synthesis of Teneraic Acid via Cobalt-Catalyzed Hydroformylation–Intramolecular Amidocarbonylation

The reaction condition of amidocarbonylation is identical to that of a hydroformylation reaction (i.e., CO–H₂ = 1 : 1, approximately 13.7 MPa, 100°C). Thus, it is possible to use N-alkenyl amides as starting materials to synthesize N-acyl-cyclic-imino acids via tandem carbonylation (hydroformylation of an olefin followed by an intramolecular amidocarbonylation). First, this method was applied to the synthesis of teneraic acid as shown in Chart 2. This synthesis constitutes a superior process because the desired carbon framework is formed in a single step reaction. Cobalt-catalyzed amidocarbonylation of (S)-N-benzoylallylglycine (12) results in a complex mixture that includes N-benzoyl teneraic acid (18) as a major product. Specifically, it is considered that the hydrogenation of olefin moiety forms N-benzoyl-norvaline (22%). The mixture was treated with ethereal diazomethane (CH₂N₂) and carefully purified by using silica gel column chromatography to obtain N-benzoyl-teneraic acid dimethyl ester (19) in a 62% isolated yield. The formation of N-benzoyl-teneraic acid should initiate the formation of alkyl cobalt intermediate 13, and this is followed by carbonylation and intramolecular amidocarbonylation via
intermediates 14, 15, 16, and 17 as previously discussed. A previous study indicated that hydroformylation of a terminal olefin proceeded regioselectively under these conditions to yield a straight-chain aldehyde, such as intermediate 14, which subsequently produced an internal N-α-hydroxyl amide (15) in situ.\(^{(15)}\) This constitutes the reason for the selective formation of the 6-membered ring selectivity.

Interestingly, normal phase HPLC analysis of obtained N-benzoyl-teneraic acid dimethyl ester (19) revealed that the trans-isomer was obtained predominantly (analytical conditions 2). The mechanism for the significant high trans-stereoselectivity in the amidocarbonylation step is discussed in detail below. However, the chiral HPLC analysis revealed that the compound 19 approximately corresponds to a 7:3 mixture of trans-(2S,6S)-isomer (19a) and trans-(2R,6R)-isomer (19b) (analytical conditions 1, Fig. 2). Furthermore, approximately 1:1 ratio of trans-(2S,6S)-isomer (19a) and trans-(2R,6R)-isomer (19b) were obtained when (RS)-N-benzoyl-allylglycine was used as a starting material. These results indicate that the reaction using (S)-N-benzoyl-allylglycine (12) proceeded selectively to form trans-(2S,6S)-isomer as a major product although minor trans-(2R,6R)-isomer was produced via epimerization at C2 asymmetric center at an early stage of the reaction. The formation of trans-(2R,6R)-isomer (19b) can be attributed to the facile migration of cobalt carbonyl moiety from δ-position (13) to α-position (20) of the amide group and to their equilibrium. This type of isomerization of an alkyl cobalt complex was previously observed in the cobalt-catalyzed isomerization–carbonylation reaction of N-acyl unsaturated cyclic amines.\(^{(16)}\)

**Synthesis of Teneraic Acid via Anodic Oxidation–Cobalt-Catalyzed Carbonylation**

An extant study already discussed stereoselective synthesis of methyl trans-N-benzoyl-6-methylpipecolate.\(^{(18)}\) The synthesis consists of regioselective anodic oxidation of N-benzoyl-6-methylpiperdine to yield N-benzoyl-α-methoxy-6-methylpiperidine, and this is followed by its subsequent stereospecific cobalt-catalyzed carbonylation. This synthesis was applied to methyl N-benzoyl-pipecolate (21) to obtain N-benzoyl-teneraic acid dimethyl ester (19) as shown in Chart 3. This synthesis method directly passes through to form a more stable alkyl cobalt complex, namely 23, and thus it is possible to avoid epimerization as observed in the fore-mentioned synthesis.

The anodic oxidation of methyl (S)-N-benzoyl-pipecolate (21) proceeded regioselectively to result in methyl (S)-N-benzoyl-α-methoxy-pipecolate (22) in good yield.\(^{(20–22)}\) Compound 22 corresponds to the N-α-hydroxyalkyl amide intermediate (7) in Chart 1 as well as the internal N-α-hydroxyalkyl

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**Fig. 2. Chiral HPLC Chromatogram of trans-(2S,6S)-N-Benzoyl-teneraic Acid Dimethyl Esters (19a) and trans-(2R,6R)-N-Benzoyl-teneraic Acid Dimethyl Esters (19b) Obtained by Hydroformylation–Intramolecular Amidocarbonylation**

**Chart 3. Synthesis of Teneraic Acid via Anodic Oxidation–Carbonylation**

Reagents: (a) anodic oxidation: Et₄N’OTs⁻ in CH₃OH; (b) CO/H₂ (13.7 MPa), Co₂(CO)₈ in acetone; (c) CH₂N₂ in CH₃OH; (d) conc. HCl, 110°C; (e) NaOCH₃ in CH₃OH.

Chart 3. Synthesis of Teneraic Acid via Anodic Oxidation–Carbonylation
amide intermediate (15) in Chart 2.

The cobalt-catalyzed carboxylation (CO–H\textsubscript{2}=1:1, approximately 13.7 MPa, 100°C) of compound 22 yielded a mixture of trans-(2S,6S)-N-benzoyl-teneraic acid dimethyl ester (19a, 36.7%), i.e., a product generated by nucleophilic opening of the complex by MeOH, and its half-ester (25, 26.3%), i.e., a product generated by nucleophilic opening of the complex by water, with a combined yield of 63.0%. The configuration of trans-(2S,6S) of compound 25 was confirmed by converting it to trans-(2R,6R)-isomer (19b) and cis–meso-isomer (19c) by treating it with sodium methoxide (CH\textsubscript{3}ONa) in methanol (Chart 3). The stereoisomers were used as a mixture of standards for chiral HPLC analysis as well as for normal phase HPLC analysis to differentiate between the isomers and to identify the stereochemistry of reaction products. The trans-configuration of compound 19a was reconfirmed by both HPLC analysis conditions by comparing its retention times with those of cis–meso-stereoisomer (19e) derived from pyridine-2,6-dicarboxylic acid (26) as described below (Figs. 3, 4). Finally, the stereochemistry of compound 19a was established by converting it to trans-(2S,6S)-teneraic acid (1) via acidic hydrolysis (Chart 3).

**Synthesis of cis–meso-Teneraic Acid** Furthermore, cis–meso-isomer of teneraic acid (3) was prepared according to the reported procedure by PtO\textsubscript{2} catalyzed hydrogenation of pyridine-2,6-dicarboxylic acid (26) as shown in Chart 4.\textsuperscript{21} Subsequently, compound 3 was converted to cis–meso-N-benzoyl teneraic acid dimethyl ester (19e) by methyl esterification that was followed by N-benzoylation. The obtained cis–meso-isomer 19c was identical to that obtained by the epimerization of compound 19a with respect to a normal phase HPLC analysis (Fig. 4).

**A Potential Mechanism for Stereoselective Carboxylation** It is possible to rationalize the cause of high selectivity in the above mentioned two syntheses by a potential mechanism shown in Chart 5.\textsuperscript{30}\textsuperscript{31} Given that HCo(CO)\textsubscript{4} (which corresponds to the actual catalyst in the carboxylation) is as strongly acidic as hydrochloric acid in a polar solvent, it is reasonable to assume that the reaction proceeds through the N-acyl-iminium ion intermediates 23a and/or 23b. The severe steric hindrance to 1,2-diequatorial substitution in 23b requires that the methoxycarbonyl group of the N-acyl-iminium intermediate should be axial in a manner similar to 23a. It is postulated that the stereo-determining step corresponds to the formation of the amidoalkyl cobalt complex 23. As shown in Chart 5, an attack of [Co(CO)\textsubscript{4}]\textsuperscript{−} with respect to the N-acyl-iminium ion 23a could occur preferentially from the less hindered equatorial side to exclusively yield the trans-N-benzoyl-teneraic acid dimethyl ester. This type of process was favored even though the cis–meso-isomer could be thermodynamically more stable.

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**Fig. 3. Chiral HPLC Chromatogram of trans-(2S,6S)-N-Benzoyl-teneraic Acid Dimethyl Ester (19a) Obtained by Anodic Oxidation–Cobalt-Catalyzed Carboxylation**

(a) trans-(2S,6S)-Isomer (19a) is initially obtained from the reaction. (b) trans-(2S,6S)-Isomer (19a) is obtained by methyl esterification of a half-ester of trans-(2S,6S)-isomer (25).

**Fig. 4. Normal Phase HPLC Chromatogram of Stereoisomers of N-Benzoyl-teneraic Acid Dimethyl Ester (19)**

(a) Mixture of cis–meso-isomer (19e), trans-(2S,6S)-isomer (19a), and trans-(2R,6R)-isomer (19b) obtained by epimerization of trans-(2S,6S)-isomer (19a). (b) trans-(2S,6S)-Isomer (19a).

Reagents: (a) PtO\textsubscript{2} in 50% acetic acid, H\textsubscript{2} (10 MPa); (b) SOCl\textsubscript{2} in CH\textsubscript{3}OH; (c) benzoyl chloride, Et\textsubscript{3}N in THF.

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Chart 4. Synthesis of cis–meso-Teneraic Acid
Characterization of Teneraic Acid

The results indicated that physical property values (NMR spectrum and mass spectrum) of synthesized teneraic acid were in reasonable agreement with those of a natural trans-isomer. Additionally, the NMR spectrum of the synthesized teneraic acid were not consistent with those of cis-meso-isomer that was synthesized independently (Fig. 5). The melting point of the synthesized teneraic acid (235–236°C) did not coincide with that of the reported value (217–219°C), and the discrepancy of the melting points of both compounds can be attributed to the low purity of the natural product relative to the synthesized one. Moreover, the circular dichroism (CD) spectra of the synthetic trans-(2S,6S)-teneraic acid (1) and natural product exhibited the same positive Cotton effect, and thus the natural product is considered to possess the same absolute configuration as that of the trans-(2S,6S)-isomer (Fig. 6). It should be noted that the absolute configuration of naturally occurring pyrrolidin-2,5-dicarboxylic acid, which corresponds to a 5-membered-ring homolog of teneraic acid, is also known as trans-(2S,5S). Conversely, it has been reported that the configuration of the naturally occurring liner (ring opened) α-iminodicarboxylic acid, alanopine, is of the meso-(RS)-form.

Conclusion

In this study, stereoselective synthesis of trans-(2S,6S)-teneraic acid was successfully achieved with high efficiency. The synthesis consists of a regioselective anodic oxidation at the α-position of methyl N-benzoyl-pipecolate. This was followed by stereospecific cobalt-catalyzed carbonylation. Additionally, trans-(2S,6S)-stereochemistry of natural teneraic acid was reconfirmed by comparing its physical properties with those of synthetic trans-(2S,6S)-teneraic acid.
The removal of the solvent under reduced pressure was followed by dissolving the residue in AcOEt (50 mL) and filtering the insoluble material. The filtrate was extracted with 10% aqueous NaHCO₃ (100 mL), and the extract was washed with AcOEt (50 mL). The aqueous phase was acidified at pH 1 with concentrated HCl and then extracted with AcOEt (50 mL, repeated twice). The organic layer was washed with brine (50 mL), dried over MgSO₄, and concentrated to obtain crude trans-N-benzoyl-teneraic acid (18) as a viscous oil (4.2 g). The residue was esterified with ethereal CH₂N₂ and subsequently purified by silica gel column chromatography (eluted with n-hexane–AcOEt) to yield a trans-N-benzoyl-teneraic acid dimethyl ester (19) as a viscous oil (5.19 g, 17.0 mmol, 62.0%); ¹H-NMR (400 MHz, CDCl₃): δ: 1.30–2.20 (6H, m), 3.67 (6H, brs), 4.26–4.50 (2H, m), 7.33 (5H, m). ¹³C-NMR (CDCl₃): δ: 18.0, 25.5, 52.3, 55.1, 58.1, 127.1, 128.6, 130.3, 135.3, 172.1, 172.5, 174.7. IR (neat): 1740, 1650 (cm⁻¹). Field desorption (FD)-MS m/z: 305.0 (M⁺)

Synthesis of trans-(25,6S)-Teneraic Acid (1) via Anodic Oxidation–Cobalt Catalyzed Carbonylation

Methyl (S)-N-benzoyl-α-methoxy-pipecolate (22)

In the study, methyl (S)-N-benzoyl-pipecolate (21) (5.50 g, 22.2 mmol), tetraethylammonium tosylate (Et₄N²⁺OT⁻, 1.40 g, 4.64 mmol) as an electrolyte and MeOH (150 mL) as a solvent were placed into a 200-mL electrolysis cell equipped with two graphite rod anodes and two graphite rod cathodes. A constant current (1A, 32 V) was passed through a cell that was externally cooled with ice water for 9 h. The solvent was removed under reduced pressure, and the residue was dissolved in a mixed solvent of ether and AcOEt (200 mL, 1:1, v/v). The organic layer was successively washed with 5% NaHCO₃ (50 mL) and brine (50 mL), dried over MgSO₄, and then concentrated. The residue was purified by silica gel column chromatography (eluted with n-hexane–AcOEt) to obtain methyl (S)-N-benzoyl-α-methoxy-pipecolate (22) as a viscous oil (3.57 g, 12.9 mmol, 58.8%); ¹H-NMR (300 MHz, CDCl₃): δ: 1.50–2.15 (5H, m), 2.17–2.28 (0.5H, brd), 2.40–2.50 (0.5H, brd), 3.07 (1.5H, brs), 3.43 (1.5H, brs), 3.75 (3H, brs), 4.30–4.45 (0.5H, m), 4.86 (0.5H, brs), 5.40 (0.5H, brs), 5.87 (0.5H, brs), 7.40 (5H, m). IR (neat): 2950, 1735, 1640, 1440, 1405, 1205 (cm⁻¹). FD-MS m/z: 277.0 (M⁺). Electrospray ionization (ESI)-MS m/z: 277.0 (M⁺)

Trans-(25,6S)-N-Benzoyl-teneraic Acid Dimethyl Ester (Dimethyl trans-(25,6S)-N-Benzoyl-piperidine-2,6-dicarboxylate, 19a)

Specifically, methyl (S)-N-benzoyl-α-methoxy-2-piperidinecarboxylate (22) (3.57 g, 12.9 mmol) and Co₂(CO)₈ (800 mg, 2.34 mmol) in acetone (50 mL) were heated in a 100-mL stainless-steel autoclave with a 1:1 mixture of carbon monoxide and hydrogen (total pressure corresponding to 13.7 MPa that was measured at room temperature) at 100°C for 4 h. The autoclave was cooled to ambient temperature, and this was followed by purging out carbon monoxide and hydrogen. The removal of the solvent under reduced pressure was followed by dissolving the residue in AcOEt (50 mL) and filtering the insoluble material. The filtrate was extracted with 10% aqueous NaHCO₃ (100 mL), and the extract was washed with AcOEt (50 mL). The combined organic phase was washed with brine (50 mL), dried over MgSO₄, and then concentrated. The residue was purified by silica gel column chromatography (eluted with n-hexane–AcOEt) to obtain trans-(25,6S)-N-benzoyl-teneraic acid dimethyl ester (19a) as a viscous oil (1.45 g,
The aqueous phase was acidified at pH 1 with concentrated HCl and then extracted with AcOEt (50 mL, repeated twice). The organic layer was washed with brine (50 mL), dried over MgSO₄, and then concentrated to yield a crude half-ester (25). The residue was esterified with ethereal CH₂N₂ and purified by silica gel column chromatography (eluted with n-hexane–AcOEt) to obtain trans-(2S,6S)-N-benzyloxy-acid di-methyl ester (19a) as a viscous oil (1.0 g, 3.39 mmol, 26.3%); ¹H-NMR (300 MHz, CDCl₃) δ: 1.35–2.30 (6H, m), 3.73 (6H, brs), 4.56 (1H, brs), 5.98 (3H, m). ¹³C-NMR (CDCl₃) δ: 18.0, 25.5, 52.3, 55.1, 58.1, 127.1, 128.6, 130.3, 135.3, 172.1, 172.5, 174.7. IR (neat): 2950, 1735, 1650, 1440, 1380, 1325, 1200, 1150, 1095, 1070, 980 (cm⁻¹). FD-MS m/z: 305.0 (M⁺). ESI-MS m/z: 274.0 (M−31). FAB-MS m/z: 306.1347 (M+H) (Calcd for C₁₀H₁₂NO₅: 306.1342). Specific rotation: [α]D²⁰ = −123.8 (c=1.0, CH₂OH).

cis-meso-Terenic Acid (cis-meso-Piperidine-2,6-dicarboxylic Acid, 1) Furthermore, trans-(2S,6S)-N-benzyloxy-acid di-methyl ester (19a) (1.10 g, 3.60 mmol) was dissolved in 6 N HCl (50 mL) and heated at 110°C for 9 h. The resulting solution was successively washed with ether (50 mL) and AcOEt (50 mL) and then concentrated under reduced pressure to obtain crude trans-(2S,6S)-piperidine-2,6-dicarboxylic acid (I) as a solid (750 mg). A solution of crude 1-HCl (750 mg) in ion-exchange water (10 mL) was applied to a cation-exchange resin (DOWEX 1×8 in the acetate form) column (100 mL) for purification. The column was washed with ion-exchange water (200 mL). Subsequently, compound 1 was eluted with aqueous 2 N acetic acid (300 mL). The eluates containing 1 were pooled and concentrated in vacuo. The residue was dissolved in water (5 mL), and activated carbon (200 mg) was added to the solution. The solution was stirred for 1 h and then filtered. The filtrate was concentrated in vacuo to obtain trans-(25S,6S)-terenic acid (I) as a white solid (465 mg, 2.70 mmol, 74.7%); ¹H-NMR (400 MHz, CDCl₃) δ: 1.35–2.30 (6H, m), 3.73 (6H, brs), 4.33 (1H, brs), 5.78 (3H, m). ¹³C-NMR (CDCl₃) δ: 18.0, 25.5, 52.3, 55.1, 58.1, 127.1, 128.6, 130.3, 135.3, 172.1, 172.5, 174.7. IR (neat): 2950, 1735, 1650, 1440, 1380, 1325, 1200, 1130, 1095, 1070, 980 (cm⁻¹). FD-MS m/z: 305.0 (M⁺). ESI-MS m/z: 274.0 (M−31). FAB-MS m/z: 306.1347 (M+H) (Calcd for C₁₀H₁₂NO₅: 306.1342).

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Conflict of Interest All authors were employees of Ajinomoto Co., Inc. when the study was conducted and have no further conflicts of interest to declare.

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