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Long-Chain Fatty Acids Elicit a Bitterness-Masking Effect on Quinine and Other Nitrogenous Bitter Substances by Formation of Insoluble Binary Complexes

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Supporting Information

ABSTRACT: We have previously found that fatty acids can mask the bitterness of certain nitrogenous substances through direct molecular interactions. Using isothermal titration calorimetry, we investigated the interactions between sodium oleate and 22 bitter substances. The hydrochloride salts of quinine, promethazine, and propranolol interacted strongly with fatty acids containing 12 or more carbon atoms. The ^{1}H NMR spectra of these substances, obtained in the presence of the sodium salts of the fatty acids in dimethyl sulfoxide, revealed the formation of hydrogen bonds between the nitrogen atoms of the bitter substances and the carboxyl groups of the fatty acids. When sodium laurate and the hydrochloride salt of quinine were mixed in water, an equimolar complex formed as insoluble heterogeneous needlelike crystals. These results suggested that fatty acids interact directly with bitter substances through hydrogen bonds and hydrophobic interactions to form insoluble binary complexes that mask bitterness.

KEYWORDS: *fatty acids, bitter substances, bitterness masking, binary complex, quinine*

INTRODUCTION

Taste comprises five basic modalities: sweetness, sourness, bitterness, saltiness, and umami. Bitter and sour tastes are generally undesirable,[1] and in particular, bitter tastes are instinctively avoided because toxic substances often taste bitter.[2] However, some physiologically beneficial species such as flavonoids, polyphenols, and pharmaceutical agents often taste bitter.[2] In food processing, bitter tastes can also be generated during fermentation and heating.[3,4] Therefore, bitterness masking needs to be considered in food preparation and pharmaceutical manufacturing.

Several bitterness-masking methods have been developed such as adding other tastants and flavors to suppress bitter tastes.[5−7] Bitterness can also be masked by applying antagonists for the bitter taste receptors, T2Rs.[8−11] Coating and encapsulating are often used in the pharmaceutical industry to mask the bitterness of drugs.[12−20] Cyclodextrin can incorporate various substances to form an inclusion complex.[21−23] Amino acid derivatives are one example of low-molecular-weight bitterness-masking compounds.[24] Zinc can also mask the bitterness of quinine, tetralone, and denatonium benzoate.[25] In many cases, however, the masking mechanisms of these compounds have not been elucidated. For food processing, these compounds must be harmless, so identifying safe bitterness-masking agents originating from foods is a desirable objective.

In a previous study,[26] we found that certain cheeses contained bitterness-masking substances. Their masking effect was attributed to the presence of fatty acids that could mask the bitterness of some substances through direct interactions. However, there was some specificity in the observed effects; for example, the bitterness of quinine was suppressed, whereas the bitterness of caffeine was not.

We will investigate the bitterness-masking mechanism of fatty acids by defining the interactions between the sodium salts of fatty acids and bitter substances using isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy. Initially, we will use ITC to screen various bitter substances to determine which of them interact with the sodium salts of fatty acids. Sodium oleate will be used for the screening because it has been shown to have the strongest bitterness-masking activity against quinine hydrochloride (QHCl) in the sensory test.[26] We will then determine the strength and mode of interactions between various sodium salts of fatty acids and the screened bitter substances using ITC. Finally, the ^{1}H NMR spectra of the bitter substances will be obtained in the absence and presence of the sodium salts of fatty acids to elucidate the interactions with the bitter substances.

The aim of this study is to elucidate which substructures of fatty acids and bitter substances interact with each other as well as their state after the interaction. These findings will lead to clarification of the bitterness-masking mechanism on a

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molecular basis. From this information, molecular models composed of fatty acids and bitter substances can be constructed to elucidate the mode of interaction.

MATERIALS AND METHODS

Chemicals. These were obtained from commercial sources. Hexanoic, octanoic, decanoic, linoleic, and linolenic fatty acids as well as sodium thiocyanate, taurine, acesulfame K, saccharin sodium dehydrate, caffeine, thiamine hydrochloride, and amygdalin were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan); lauric acid, *N*-phenylthiocarbamide, salicin, berberine hydrochloride monohydrate, and quinine hydrochloride dihydrate were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). The myristoleic, palmitoleic, oleic, and arachidonic fatty acids as well as glycerol monooleate, (±)-propranolol hydrochloride, promethazine hydrochloride, chloramphenicol, yohimbine hydrochloride, colchicine, cromolyn disodium salt, limonin, and ouabain octahydrate were obtained from Sigma-Aldrich Co. (Tokyo, Japan). Methyltryptophan and naringin were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Acetic acid and antipyrine were obtained from Kanto Chemical Co. Inc. (Tokyo, Japan), and glycerol monolaurate was obtained from Chem-Implex International, Inc. (Wood Dale, IL, USA).

Screening of Bitter Substances. ITC was carried out using a MicroCal iTC200 instrument (Malvern Instruments Japan Corp., Kobe, Japan). The fatty acids were neutralized and solubilized by adding an equimolar amount of sodium hydroxide. The interactions between 22 bitter substances, whose structures are shown in Figure S1-1, and sodium oleate were measured. The concentrations of the bitter substances and sodium oleate were 1.5 and 0.5 mM, respectively. The compounds were dissolved in 5 mM phosphate buffer (pH 7.0). The reference cell was filled with Milli-Q water (Millipore Corp., Billerica, MA, USA). The bitter substances in buffer solutions were titrated into sodium oleate dissolved in 5 mM phosphate buffer (pH 7.0) at a stirring rate of 1000 rpm and at 25 °C. Each titration was carried out with an initial injection volume of 0.4 μL, followed by 18 main injections of 2 μL each at 120 s intervals. The first titration (0.4 μL) was excluded from the analysis. The data corresponding to the dilution of the ligand in the buffer was subtracted from the titration data.

Measurement of Interactions between Fatty Acids and Bitter Substances. The interactions between five bitter substances, QHCl, promethazine hydrochloride (PHCl), propranolol hydrochloride (PrHCl), berberine hydrochloride (BHCl), and yohimbine hydrochloride (YHCl), and sodium salts of eight fatty acids (sodium hexanoate, sodium octanoate, sodium decanoate, sodium laurate, sodium myristoleate, sodium oleate, sodium linoleate, and sodium linolenate) and two monoacylglycerides (glycerol monolaurate and glycerol monooleate) were measured. The concentrations of the fatty acids were 0.5 mM, and those of the bitter substances are given in Table S1. The samples were dissolved in 5 mM phosphate buffer (pH 7.0). Titrations were conducted as described above.

The data were analyzed according to the "one set of sites" model provided in Origin 7.0 software for MicroCal iTC_{200}. The binding constant (K) and enthalpy change (ΔH) of binding were obtained from the fitted curve. The entropy change (ΔS) and free energy change (ΔG) of binding were obtained using the following equation:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K$$

where R is the gas constant and T is the thermodynamic temperature.

Preparation of Crystals. QHCl (3 mmol, 1.08 g) was dissolved in 3 L of Milli-Q water. A solution of sodium laurate (3 mmol, 0.67 g) in Milli-Q water (50 mL) was added to the QHCl solution, and the mixture was thoroughly stirred. The mixture was allowed to stand overnight at room temperature. The precipitated crystals were filtered (No. 5A, Toyo Rishi Kaisha, Ltd., Tokyo, Japan), rinsed with water, and air-dried. Differential scanning calorimetry (DSC) measurements were carried out using a Shimazu DSC-60 (Kyoto, Japan) in the temperature range of 5−60 °C at a heating rate of 5 °C/min under a nitrogen atmosphere.

NMR Spectroscopy. All NMR experiments except for solid-state NMR were conducted on a Varian Inova 500 NMR spectrometer (500 MHz) at 25 °C (Varian Inc., Palo Alto, CA, USA). The bitter substances (32 μmol; QHCl, PHCl, and PrHCl) were measured in the absence and presence of sodium laurate. The samples were dissolved in 600 μL of DMSO-d_6. The ^{1}H NMR measurement conditions were as follows: number of data points: 16 384; acquisition time: 1.75 s; delay time: 5 s; and number of scans: 16. The NOESY (nuclear Overhauser enhancement spectroscopy) measurement conditions were as follows: number of data points: 512 and 1024 for F1 and F2, respectively; digital resolutions of F1 and F2: 5.50 and 8.79 Hz, respectively; delay time: 1.5 s; mixing time: 1.0 s; and number of scans: 16. Solid-state NMR was conducted using an ECA 500 (500 MHz) (JEOL, Tokyo, Japan) at 0 °C to stop the crystals from melting, using 4 mm of CP/MAS (cross-polarization/magic angle spinning) probe at 10 000 rpm. The measurement was as follows: number of data points: 1024; digital resolution: 49.2 Hz; delay time: 5.0 s; and number of scans: 2048.

Molecular Modeling. Initial models of conformations that were consistent with the NOESY data of the three bitter substances in the absence and presence of sodium laurate were manually constructed using GaussView 5.0 (Gaussian, Inc., Wallingford, CT, USA). The geometries were optimized in vacuo at the B3LYP/6-31G (d) level of theory; then, the geometries were optimized in DMSO at the same level of theory. The polarizable continuum model (PCM) was used to calculate solvent effects in DMSO. All calculations were carried out using the Gaussian 09 software package.[27]

RESULTS

ITC Measurements. The interactions between 22 bitter substances and sodium oleate were studied using ITC (Figure S1-2). Five bitter substances, QHCl, PHCl, PrHCl, BHCl, and YHCl were found to interact with sodium oleate. Notably, each of these substances bears a nitrogen atom (Figure 1). The

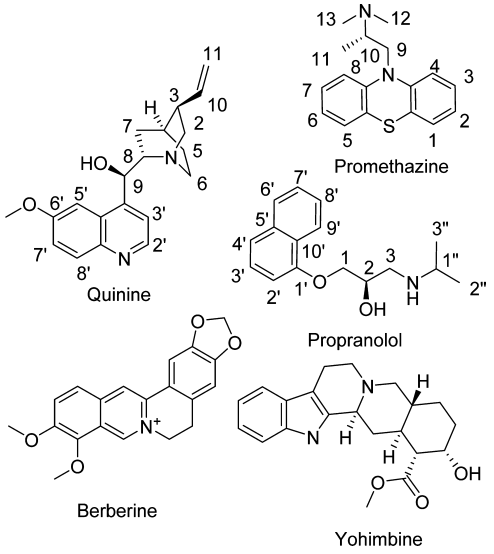

Figure 1. Chemical structures of five bitter substances showing exothermic interactions with sodium oleate.

interactions between these five substances and six fatty acids were then studied (Table 1). The change in Gibbs free energy (ΔG) was negative for all the interactions detected, meaning that these interactions occurred spontaneously. The change in enthalpy (ΔH) contributed to ΔG in all cases, and the change in entropy (ΔS) contributed in some cases (Table 1). In these

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Table 1. Thermodynamic Parameters for Association between Five Bitter Substances and Sodium Salts of Fatty Acids

		QHCl	PHCl	PrHCl	BHCl	YHCl
lauric acid (12:1)	ΔH[a]	−25.5 ± 3.5	−20.3 ± 2.6	−16.7 ± 2.3	n.d.[b]	n.d.
	$T\Delta S$[c,d]	−3.5 ± 3.5	0.7 ± 2.6	1.3 ± 2.5	n.d.	n.d.
	ΔG[e]	−22.0 ± 0.1	−21.0 ± 0.0	−18.0 ± 0.1	n.d.	n.d.
myristoleic acid (14:1)	ΔH	−10.8 ± 1.4	−12.6 ± 0.7	n.d.	n.d.	n.d.
	$T\Delta S$	13.7 ± 1.9	11.0 ± 0.6	n.d.	n.d.	n.d.
	ΔG	−24.5 ± 0.5	−23.6 ± 0.1	n.d.	n.d.	n.d.
palmitoleic acid (16:1)	ΔH	−28.0 ± 2.6	−21.8 ± 2.5	−24.4 ± 1.4	n.d.	n.d.
	$T\Delta S$	−0.3 ± 2.8	5.2 ± 3.0	1.3 ± 1.5	n.d.	n.d.
	ΔG	−27.6 ± 0.3	−27 ± 0.6	−25.7 ± 0.1	n.d.	n.d.
oleic acid (18:1)	ΔH	−17.7 ± 0.4	−10.7 ± 0.2	−6.4 ± 0.1	−11.5 ± 0.2	−10.0 ± 0.5
	$T\Delta S$	11.5 ± 0.4	18.3 ± 0.3	22.0 ± 0.1	15.7 ± 0.2	14.6 ± 0.4
	ΔG	−29.2 ± 0.0	−29.0 ± 0.3	−28.4 ± 0.3	−27.2 ± 0.0	−24.6 ± 0.2
linoleic acid (18:2)	ΔH	−27.3 ± 3.1	−21.0 ± 2.3	−28 ± 1.7	n.d.	n.d.
	$T\Delta S$	1.3 ± 3.1	7.5 ± 2.4	−2.1 ± 1.8	n.d.	n.d.
	ΔG	−28.6 ± 0.1	−28.6 ± 0.1	−25.8 ± 0.1	n.d.	n.d.
linolenic acid (18:3)	ΔH	−42.3 ± 2.5	−30.4 ± 3.2	−28.2 ± 3.8	−34.5 ± 0.8	−7.8 ± 1.5
	$T\Delta S$	−15.1 ± 2.5	−2.6 ± 3.2	−2.6 ± 4.0	8.8 ± 8.2	−17.4 ± 1.7
	ΔG	−27.2 ± 0.0	−27.8 ± 0.0	−25.6 ± 0.2	−25.6 ± 0.1	−25.1 ± 0.3

[a] ΔH, enthalpy change (kJ/mol). [b] n.d., not determined by calculation according to "one set of site" model using the titration curve. Mean and standard deviation values are obtained from the fitted data of three independent experiments. [c] T, thermodynamic temperature (K). [d] ΔS, entropy change (kJ/(mol K)). [e] ΔG, Gibbs free energy (kJ/mol).

Table 2. Binding Constant K ($\times 10^3$ M^{-1}) Derived from ITC Profiles for the Association between Five Bitter Substances and Nine Sodium Salts of Fatty Acids and Two Glycerol Esters

	QHCl	PHCl	PrHCl	BHCl	YHCl
hexanoic acid (6:0)	n.d.[a]	n.d.	n.d.	n.d.	n.d.
octanoic acid (8:0)	n.d.	n.d.	n.d.	n.d.	n.d.
decanoic acid (10:0)	n.d.	n.d.	n.d.	n.d.	n.d.
lauric acid (12:0)	7.2 ± 0.2	4.9 ± 0.0	1.4 ± 0.1	n.d.	n.d.
myristoleic acid (14:1)	19.9 ± 4.0	13.7 ± 0.3	n.d.	n.d.	n.d.
palmitoleic acid (16:1)	69.2 ± 6.9	54.8 ± 14.0	32.2 ± 2.2	n.d.	n.d.
oleic acid (18:1)	129 ± 1.2	123.0 ± 14.3	94.8 ± 9.1	59.5 ± 4.8	20.4 ± 1.5
linoleic acid (18:2)	101.1 ± 3.4	100.7 ± 4.9	33.4 ± 0.8	n.d.	n.d.
linolenic acid (18:3)	60.4 ± 0.7	74.2 ± 0.5	31.2 ± 2.2	31.1 ± 1.0	25.8 ± 3.6
glycerol monolaurate	n.d.	n.d.	n.d.	n.d.	n.d.
glycerol monooleate	n.d.	n.d.	n.d.	n.d.	n.d.

[a] n.d., not determined by calculation according to the "one set of site" model using the titration curve. Mean and standard deviation values are obtained from the fitted data of three independent experiments.

cases, the titrated solutions became turbid upon injecting the bitter substances, indicating that insoluble complexes had been formed between the fatty acids and the bitter substances. Of the five bitter substances examined, BHCl and YHCl exhibited relatively weak interactions with the fatty acids (Table 2). Therefore, only the three that had strong interactions with the fatty acids were examined: QHCl, PHCl, and PrHCl.

No interactions were detected between fatty acids with 10 or fewer carbon atoms and the three bitter substances. All three substances showed the greatest binding constants upon binding with sodium oleate, which increased as the number of carbon atoms in the alkyl chain of the fatty acids increased. The binding constant also decreased as the number of double bonds in the fatty acids increased (Table 2). These results suggested that the carbon chain length and the number of double bonds in the fatty acids affected these interactions.

To examine the effect of the carboxyl group in the fatty acids, the interactions between the bitter substances and glycerol monolaurate and glycerol monooleate were also measured. However, no interactions between the bitter substances and these glycerol esters were detected (Table 2).

Overall, carbon chains of a certain length and the presence of carboxyl groups in the fatty acids were necessary to induce interactions with the bitter substances.

NMR Measurements. ITC measurements proved that fatty acids interacted directly with some bitter substances. To examine the mechanism of these interactions, the ^{1}H NMR spectra of three bitter substances (QHCl, PHCl, and PrHCl) were measured in the absence and presence of an equimolar amount of sodium laurate. Because these bitter substances are insoluble in water when sodium laurate is added, DMSO-d_6 was used as the solvent instead.

The three bitter substances contained a nitrogen atom (Figure 1). The signals of the protons on the hydrogen atoms located near the nitrogen atoms was shifted upfield in the presence of sodium laurate (H2, H6, and H8 of QHCl; H9–H13 of PHCl; and H1″ and H3 of PrHCl; Figure 2). The changes in the chemical shift ($\Delta\delta$) of these protons were more than 0.3 ppm for QHCl and PHCl and more than 0.2 ppm for

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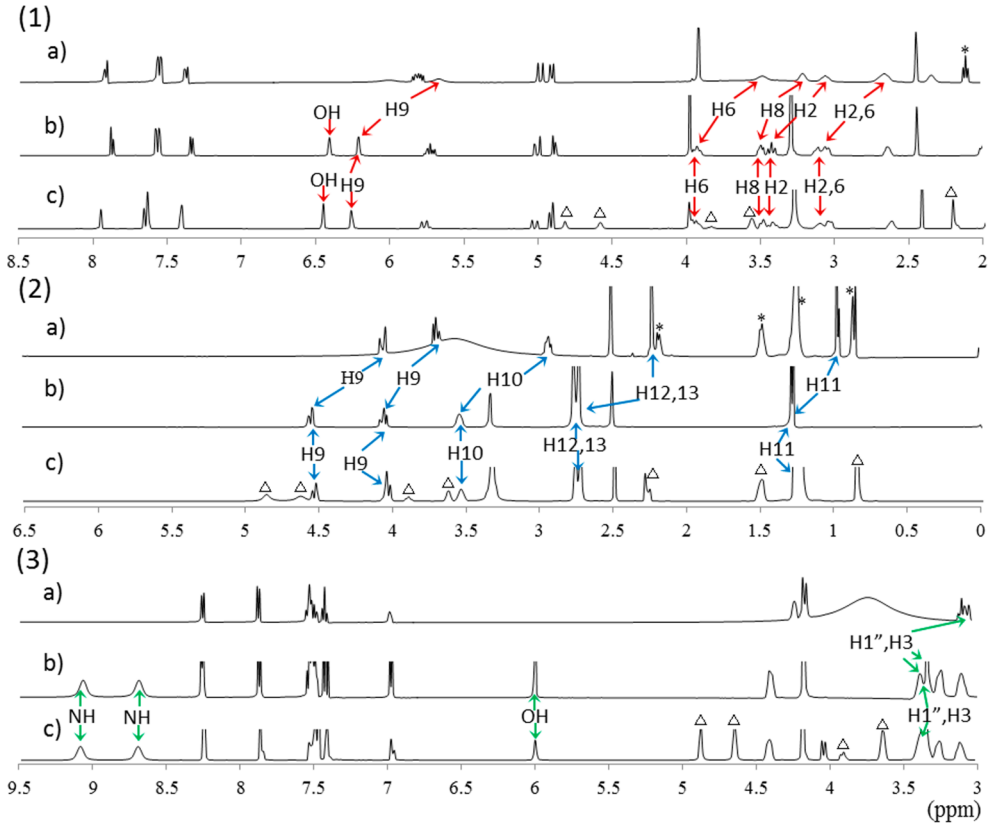

Figure 2. ^{1}H NMR spectra of (1) QHCl, (2) PHCl, and (3) PrHCl. Spectra of (a) bitter substances in the presence of sodium laurate, (b) bitter substances alone, and (c) bitter substances in the presence of glycerol monolaurate. Characteristic protons are indicated by arrows. The broadening of the OH and perturbation of H9 in QHCl and the broadening of OH and NH in PrHCl resulted from the exchange of OH or NH protons with those in the carboxyl moiety in sodium laurate. *, signals of sodium laurate; △, signals of glycerol monolaurate.

PrHCl (Table S2). In the presence of glycerol monolaurate, no significant changes were observed ($\Delta\delta$ < 0.02 ppm) (Table S3).

The ^{1}H NMR spectra of QHCl were also measured in the presence of sodium decanoate (10:0), sodium octanoate (8:0) and sodium hexanoate (6:0) which caused the signals corresponding to H2, H6, and H8 of QHCl (Table S4) to be shifted upfield ($\Delta\delta$ > 0.3 ppm). These results suggested that the hydrogen bond between the nitrogen atom of the bitter substances and the carboxyl group of the fatty acid was formed in DMSO.

In addition, the ^{1}H NMR spectra were measured after changing the molar ratio of sodium laurate to QHCl (0, 0.2, 0.5 and 0.8). The changes in the chemical shifts of H2, H6, and H8 ($\Delta\delta$) were plotted as a function of the molar ratio of sodium laurate (Figure 3). As the chemical shifts depended on the molar ratio of sodium laurate, the exchange between the free and complexed states was faster than the chemical shift difference between the two states.

ITC measurements revealed that the length of the carbon chain and the number of double bonds in the fatty acids affected the binding constants, which suggested that the alkyl chains contributed to the interactions. To examine where the hydrophobic interactions occurred, the NOESY spectra of the bitter substances were measured in the absence and presence of sodium laurate (Figure 4).

In the present study, there were three possible locations for hydrophobic interactions: the first was between the fatty acids and bitter substances, the second was between different molecules of the bitter substances, and the third was between

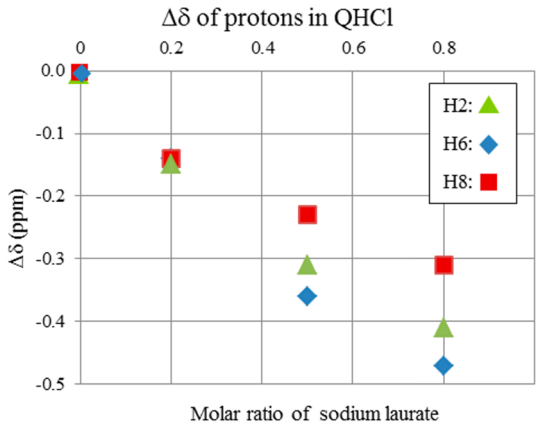

Figure 3. Titration curve corresponding to QHCl (H6, H8, and H2) upon addition of sodium laurate. The change in the chemical shift ($\Delta\delta$) in 32 μmol of QHCl is plotted as a function of the molar ratio of sodium laurate (0, 0.2, 0.5, and 0.8).

different molecules of the fatty acids. Although no cross-peaks were observed between sodium laurate and QHCl, some were observed between H2′ and H8′ of QHCl in the presence of sodium laurate (Figure 4). Because these protons cannot interact with each other within a single molecule of QHCl, intermolecular hydrophobic interactions between two molecules of QHCl occurred in the presence of sodium laurate. However, in the case of PHCl and PrHCl, no interactions between bitter substances and fatty acids and between different molecules of bitter substances were observed (Figure S3 and

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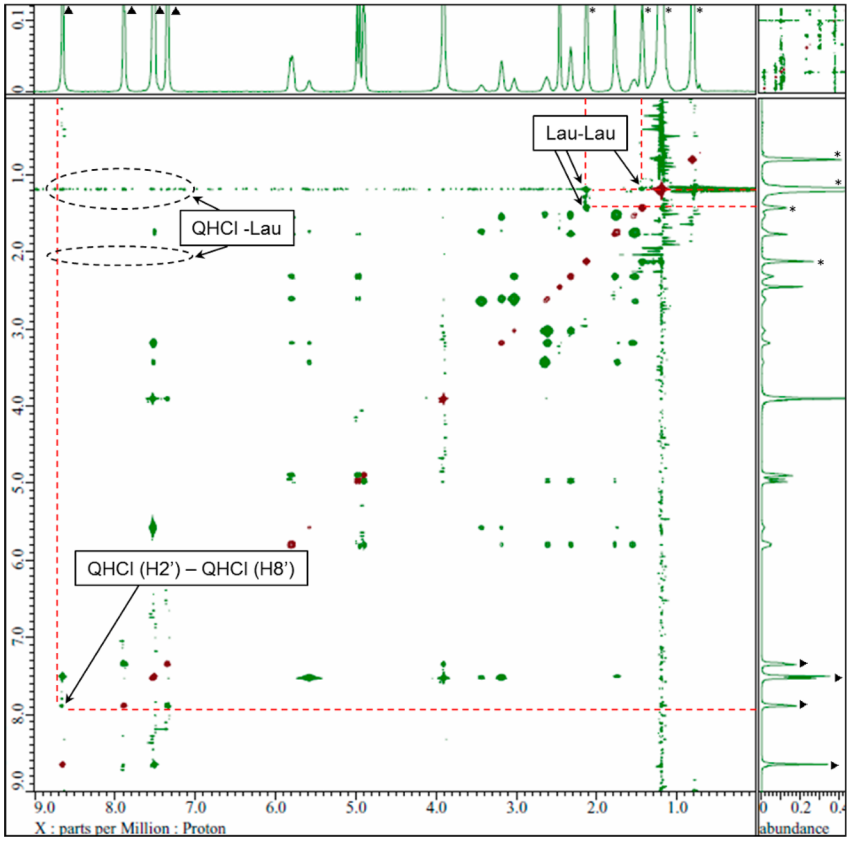

Figure 4. NOESY spectra of QHCl in the presence of sodium laurate. ^{1}H NMR spectra of QHCl/sodium laurate is displayed on X−Y axis. Characteristic cross-peaks are indicated by red circles. *, signals of sodium laurate; ▲, signals of aromatic protons in QHCl.

Table S5). Detailed analysis of the cross-peaks between carbon chains was impossible because many signals overlapped. However, because the length of the carbon chain affected the strength of the interactions in the ITC experiment, we concluded that hydrophobic interactions existed primarily between the carbon chains in an aqueous environment. On the basis of the NOESY results, we constructed molecular models of the binary complexes formed between the bitter substances and sodium laurate in DMSO (Figure 5).

Crystal Analysis of Sodium Laurate and QHCl. Needlelike crystals were observed upon mixing an aqueous solution of sodium laurate and QHCl (Figure 6a). These crystals were composed of an equimolar amount of laurate and quinine, as indicated by ^{1}H NMR. The DSC curve of the crystal revealed a wide exothermic peak (Figure 6b). From CP/MAS NMR spectra, it appeared that C2, C6, and C8 in QHCl resonated at chemical shifts of 62.3, 56.9, and 66.3 ppm, respectively, and each of the signals split into three peaks with a broadened peak width when the crystal was formed with lauric acid (Figure S2). This indicates that at least three states were involved in the crystal. In addition, peak broadening of the methyl and methylene carbon atoms in lauric acid upon formation of crystal demonstrated an incoherent microcrystalline structure. X-ray diffraction analysis was applied to the ground crystal samples using the radial distribution function $4\pi r^2\rho$ (r) to obtain structural information. The sum of the data for QHCl and lauric acid, measured independently, was different from that of the data for the ground crystal sample, implying that specific interactions had been formed in the complex. The crystal seems to be in an intermediate state regarding its amorphosity and crystal style (data not shown).

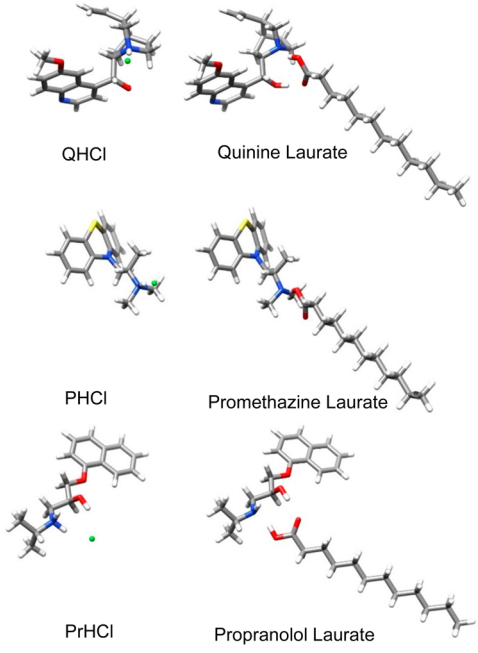

Figure 5. Structures of three bitter substances and their laurates. Atoms are presented as carbon in gray, oxygen in red, sulfur in yellow, nitrogen in blue, and chloride in light green.

These results suggested that the crystal was composed of various conformations. The bitterness of the crystal was significantly less than that of QHCl alone using sensory testing (data not shown).

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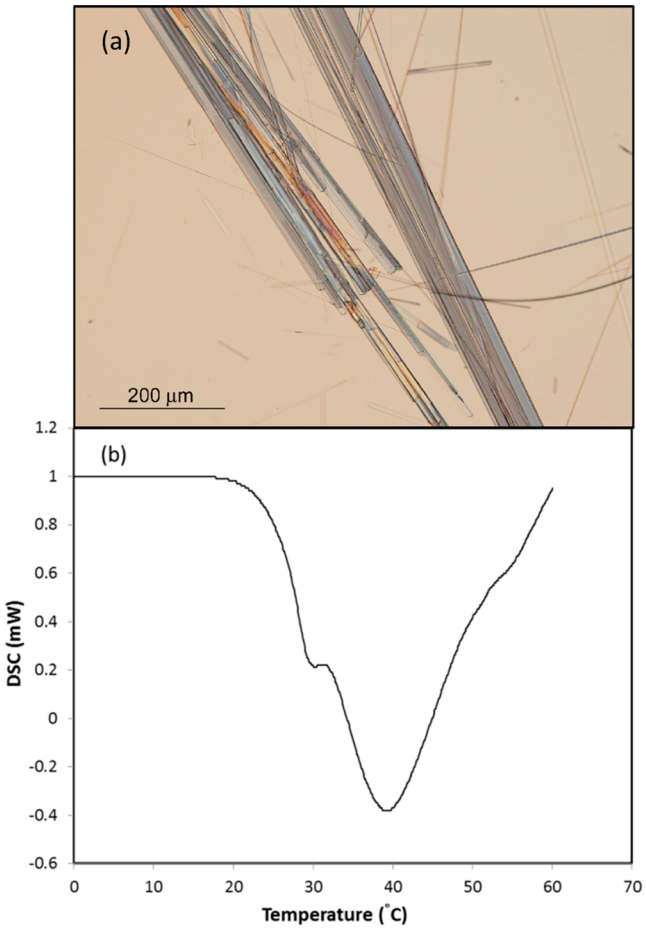

Figure 6. (a) Needlelike crystals formed between sodium laurate and QHCl. (b) DSC thermal curve of the crystal.

DISCUSSION

In a previous report,[26] it was shown that sodium oleate suppressed the bitterness of QHCl. Exothermic interactions were observed when QHCl was added to the fatty acid, associated with masking the bitterness of QHCl by direct interaction with the fatty acids. However, the detailed mechanism of the bitterness-masking reaction has not been elucidated. In the present study, we found that two types of interaction, hydrogen bonds and hydrophobic interactions, were necessary to mask the bitterness of some compounds. Specifically, NMR revealed that hydrogen bonding occurred between the nitrogen atom of the bitter substances (N1 of QHCl, N11 of PHCl, and N4 of PrHCl) and the carboxyl group of the fatty acids in DMSO (Figure 2). In addition, the ITC experiments in water indicated that the strength of the interaction between the bitter substance and the fatty acid depended on the length of the carbon chain of the fatty acid, demonstrating the involvement of hydrophobic interactions.

In aqueous solutions, the bitter substances and fatty acids dissociated as weak bases and acids, respectively.[28] In neutral pH solutions, the dissociation equilibrium of the long-chain fatty acids (RCOOH) shifted to the left of the equilibrium reaction (eq 1), given that the pK_a values of oleic acid, linoleic acid, and linolenic acid are 9.85, 9.24, and 8.28, respectively.[28]

$$\mathrm{RCOOH} \leftrightarrows \mathrm{RCOO^-} + \mathrm{H^+} \quad (1)$$

However, the dissociation equilibrium of bitter nitrogenous substances (BN) such as quinine, promethazine, and propranolol shifted to the left side of the equilibrium reaction (eq 2), given that the pK_a values of quinine,[29] promethazine,[30] and propranolol[31] are 8.3, 8.6, and 9.2, respectively.

$$\mathrm{BNH^+} \leftrightarrows \mathrm{BN} + \mathrm{H^+} \quad (2)$$

The ΔH values for the interactions between the fatty acids and bitter substances were in the range of 10−40 kJ/mol in all cases (Table 1). These results suggested that hydrogen bonds existed between the fatty acids and bitter substances. Two possible mechanisms of hydrogen bond formation are proposed: One involves interaction between RCOOH and BN that comprises a unit of the binary complex, and the other interaction occurs between BH^+ and $RCOO^-$. The former hypothesis can explain the observation that long-chain fatty acids interact with the bitter substances whereas short-chain fatty acids do not. The latter hypothesis can account for the ionic attraction between oppositely charged species.

Of the 22 bitter substances examined, those that exhibited strong interactions with the fatty acids contained secondary or tertiary amino groups. Although some bitter substances that did not interact with the fatty acid also had nitrogen atoms, most of these nitrogen atoms were present in amide groups or in conjugated systems. The rest were present in primary amino groups that might be unsuitable for forming hydrophobic interactions with the carbon chain of the fatty acids.

Regarding hydrophobic interactions, the contribution of the carbon chains was obvious because the length of the carbon chain and the number of double bonds affected the binding constants (Table 2). The NOESY data indicated the possibility of a stacking effect between the aromatic rings of QHCl. However, the $T\Delta S$ values varied widely and were not correlated with ΔG, especially with sodium linoleate (Table 1). This phenomenon was perhaps due to either the conformational variation of the fatty acids or the decreased solubility of the mixture of hydrochloride salts of the bitter substances and the sodium salts of the fatty acids.

On the basis of these results, we propose that the bitterness of substances can be masked with fatty acids by the formation of a binary complex between these species through a hydrogen bond and a hydrophobic interaction (Figure 7). Specifically, the aggregation of a unit of binary complex of fatty acids and bitter substances generated large insoluble complexes. The formation of these complexes made it more difficult for the bitter substances to interact with the bitter taste receptors, thereby masking the bitterness. Particularly in the case of QHCl and sodium laurate, both substances aggregated because of hydrophobic interactions between the carbon chains, forming needlelike crystals (Figure 7). As suggested by DSC, the crystal was probably composed of quinine laurate in various conformations with stacking interactions between the aromatic rings of QHCl.

In the present study, fatty acids have been found to interact directly with bitter substances through hydrogen bonds and hydrophobic interactions. Moreover, both types of interaction played important roles in masking bitterness. This study has been described how direct interactions between bitterness-masking substances and bitter substances can suppress bitterness. The results of this study should lead to the development of new method for masking bitterness, which may be directly applicable to food processing. More research would be needed to investigate the taste and acceptability of

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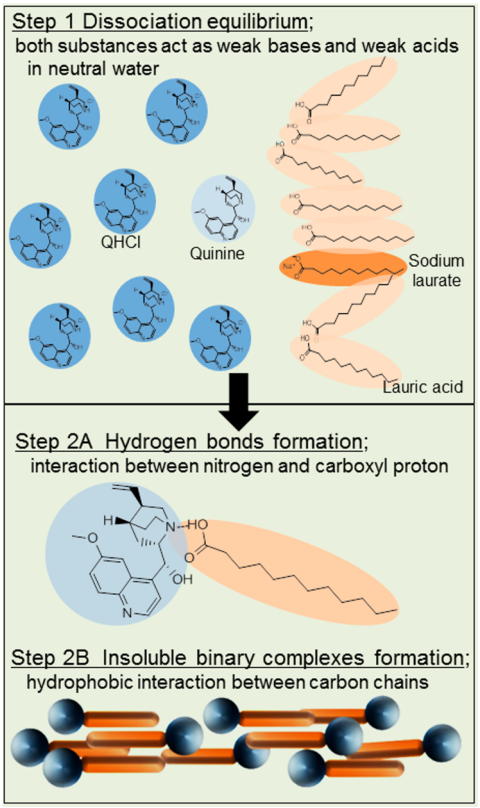

Figure 7. Possible mechanism of interaction between sodium laurate and QHCl. Sodium laurate and QHCl interact weakly via step 1, comprising dissociation equilibrium of QHCl as weak bases and sodium laurate as weak acids. Hydrogen bonding and insoluble binary complexes occur via step 2; for fatty acids with a chain length of 12 or more carbon atoms, hydrogen bonds form between quinine and laurate. The complex model is presented in the lower image. QHCl molecules are colored blue, and sodium laurate is shown in orange. The binary complexes are connected to each other by hydrophobic interactions and grow in a single direction, forming needlelike crystals.

food containing fatty acids and nitrogen-containing bitter compounds.

From this study, we can propose a possible mechanism for how fatty acids mask the bitterness of bitter substances. Both hydrogen bonds and hydrophobic interactions are essential for forming the insoluble binary complexes composed of bitter substances and fatty acids. Nitrogen atoms in the bitter substances and fatty acids with chain lengths of 12 or more carbon atoms are necessary for forming the binary complexes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b03193.

The structures of screened substances and the isothermal titration profiles, solid-state NMR spectra of crystal, lauric acid and QHCl, ^{1}H NMR assignments of QHCl, PHCl and PrHCl in the absence and presence of sodium laurate, ^{1}H NMR assignments of QHCl, PHCl and PrHCl in the absence and presence of glycerol monolaurate, ^{1}NMR assignments of QHCl in the absence and presence of sodium decanoate, sodium octanoate and sodium hexanoate, NOESY assignments of QHCl, PHCl and PrHCl in the absence and presence of sodium laurate, NOESY spectra of PHCl and PrHCl in the presence of sodium laurate. (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ITC, isothermal titration calorimetry; ^{1}H NMR, proton nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; DMSO, dimethyl sulfoxide; T2Rs, taste receptors type 2; K, binding constant; ΔH, enthalpy change of binding; ΔS, entropy change of binding; ΔG, free energy change of binding; R, the gas constant; T, thermodynamic temperature; QHCl, quinine hydrochloride; PHCl, promethazine hydrochloride; PrHCl, propranolol hydrochloride; BHCl, berberine hydrochloride; YHCl, yohimbine hydrochloride; CP/MAS, cross-polarization/magic angle spinning; DSC, differential scanning calorimetry; PCM, polarizable continuum model

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