

Hydrogen Bonding in Alcoholic Beverages (Distilled Spirits) and Water–Ethanol Mixtures

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The hydrogen-bonding properties of water–ethanol of alcoholic beverages and water–ethanol mixtures of the corresponding ethanol contents were examined on the basis of OH proton NMR chemical shifts and the Raman OH stretching spectra of water and ethanol. Japanese *shochu*, an unaged distilled spirit of 25% (v/v) alcoholic content made from various grains, was provided for the samples; it is a high-purity spirit as it contains only small amounts of dissolved components, like typical vodka, gin, and white rum. The hydrogen-bonding structure in *shochu* containing some acids was found to be different from that of the water–ethanol mixture with corresponding ethanol content. It was concluded that, by the presence of small amounts of organic acids, the water–ethanol hydrogen-bonding structure was strengthened, at the same time, the proton exchange between water and ethanol molecules was promoted in *shochu*, compared with the water–ethanol mixture. The NMR chemical shifts of fruit cocktail drinks suggested that the hydrogen bonding of water–ethanol in the solution was developed by organic acids and (poly)phenols from fruit juices.

KEYWORDS: Chemical shift in ¹H NMR; Raman OH stretching; hydrogen bond; water structure; ethanol; cocktail; *shochu*; Japanese sake

INTRODUCTION

Water occupies the largest part of alcoholic beverages. In water–ethanol solutions, the interaction through hydrogen bonding should operate with water–water, water–ethanol, and ethanol–ethanol molecules. In the manufacturing process, whiskey and other distilled spirits are usually matured in wood casks for several years or more. After this long maturation, the organoleptical stimulation of ethanol is reduced. The reduction of the stimulative taste of ethanol has been regarded as the change of the hydrogen-bonding structure of water–ethanol molecules (1). So far, several studies have been reported on the interaction of water–ethanol molecules and the hydrogen-bonding structure in aged spirits (2–7). We have paid attention to the relationship between the reduction of organoleptical stimulation of ethanol and the hydrogen bonding of water–ethanol of alcoholic beverages. Conner et al. (8–10) noted that organic acids or wood components played an important role in increasing the solubility of distillate compounds in aged whiskey.

In the field of solution chemistry, the study of water–alcohol solutions is one of the most attractive subjects. Franks and Ives

(11) reviewed the structural properties of the alcohol–water mixtures. Coccia et al. (12) analyzed the ¹H NMR signals in ethanol–water mixtures throughout the entire range of composition. Nishi et al. (13) examined the molecular association in ethanol–water mixtures by mass spectrometric analysis. Three-dimensional structure in methanol–water mixtures was investigated by Laaksonen et al. (14). Price et al. (15) examined the associative behavior in aqueous monohydric alcohol systems. Sato and Buchner (16) discussed the dynamics of ethanol–water systems by analyzing dielectric relaxation. Dixit et al. (17) reported the molecular scale structure of a concentrated alcohol–water mixture. In alcohol–water mixtures, the clathrate structures were also reported (18). Recently, the state of molecular associates (19) or the phase separation (20) in binary mixtures has been discussed. Fileti et al. (21) calculated the hydrogen-bonding energies for the different isomers of 1:1 complexes of methanol, ethanol, and water. Guo et al. (22) elucidated the structural properties of liquid methanol as well as of methanol–water solutions.

We have investigated the effects of solutes on the hydrogen-bonding structure of EtOH–H₂O mixtures by analyzing the ¹H NMR chemical shifts and found that the hydrogen-bonding structure of water–ethanol is strengthened by stronger hydrogen-bonding donors (acids) as well as acceptors (conjugate bases from weak acids); we have proposed a model for the intimate interaction between water and ethanol molecules (23). We

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suppose that the change of the hydrogen-bonding structure in the whole solution occurs immediately on the addition of those substances. Furthermore, the hydrogen-bonding structure was also investigated in aged whiskey (24) and Japanese sake, a kind of wine from rice (25). We were able to demonstrate that the hydrogen-bonding structure in aged whiskies is directly developed by acidic and phenolic components gained in oak wood casks and is not dependent on just the aging time (24). In Japanese sake, it was also confirmed that the hydrogen bonding is strengthened by acidic, amino acidic, and phenolic components produced by microorganisms during fermentation (25). However, sugar components, such as glucose, maltose, and sorbitol, never caused a distinct development of hydrogen bonding in water-ethanol solutions (24).

In the present study, we investigated the hydrogen-bonding structure of water-ethanol in a Japanese distilled spirit (*shochu*) by means of proton NMR and Raman spectroscopy. *Shochu* is made from various grains, usually accompanied by no aging in wood casks after distillation. A few of them are matured in wood casks for just a couple years because of the regulation (by Japanese law) regarding their darkness of color. *Awamori* (a kind of *shochu* made from Thai rice only in Okinawa Prefecture) may be matured in clay pots for more than several years. At any rate, *shochu* contains small amounts of chemical components compared with brown liquors (whiskey and brandy) or brewed beverages such as grape wine, beer, and Japanese sake. With the amount of chemical components such as acids, esters, alcohols, and inorganic ions, *shochu* is similar to white rum or vodka, regardless of ethanol contents. We examined the relationship between the hydrogen-bonding structure in *shochu* and acidic and phenolic compounds, inorganic substances, and flavor components. We focused on the difference in water-ethanol hydrogen-bonding structure between ethanol-water mixtures and *shochu*. The hydrogen-bonding conditions in cocktails (mixtures of sprits with fruit juices) will also be discussed.

MATERIALS AND METHODS

Chemicals. Commercially obtained reagents of analytical grade were used without further purification: acetic acid (99.0%, Nacalai), vanillic acid (98%, TCI), vanillin (98.0+%, Wako), ethyl caproate (Nacalai), ethyl caprylate (Nacalai), isoamyl acetate (99%, Nacalai), propanol (99.5+%, Wako), isoamyl alcohol (98+%, Wako), and C_2H_5OH (Wako, GR grade). Commercially obtained inorganic salts of analytical grade were used as received. Water was purified by means of a Milli-Q system (Millipore Corp.).

Spirit Samples. *Shochu* samples of commercial products were provided for analyses. *Shochu* is a traditional Japanese spirit, especially popular with the younger generation. This spirit has two different distilling procedures: (A) *kou* (continuous distillation) and (B) *otsu* (batch distillation). *Kou* is made from grains (corn, potato, etc.) or molasses, and *otsu* is made from individually different grains (barley, rice, sweet potato, or others). The *shochu* made from "others" is made from miscellaneous grains. Twenty-nine samples were analyzed in total. Some barley-derived *shochu* samples matured in wood casks and *awamori* (typically aged in clay pots) were included. Fruit cocktail drinks containing carbon dioxide were commercially obtained.

1H NMR Chemical Shifts. The 1H NMR chemical shifts of the hydroxyl groups in EtOH-H₂O solutions containing various compounds or alcoholic beverages were measured with a JEOL JNM-LA 400 NMR spectrometer (JEOL, Ltd., Tokyo Japan) at 25.0 ± 0.1 °C in Wilmad coaxial tubes (23). D₂O (99.9% D, Aldrich) in the inside tube served as the locking solvent. According to the method of Wen and Hertz (26), the chemical shift of the hydroxy proton resonance was determined relative to the peak due to the methyl group of EtOH: the center peak of the triplet peak of the methyl group was utilized as the standard (CH_3 1.164 versus TMS) (23-25). Mizuno et al. (27, 28) have discussed

that 1H chemical shifts of CH_3 protons are dependent on the composition of solvents. In our studies, the solute effects have been evaluated for solutions of constant EtOH-H₂O compositions as the general procedure. With TMS as the external standard, we have verified that the effects of the solutes [e.g., malic acid in 20% (v/v) EtOH-H₂O solution] on chemical shift values of CH_3 protons were negligible. A similar check was also carried out in MeOH-H₂O and *t*-BuOH-H₂O solution systems. In a MeOH-H₂O solution, the chemical shift value of the CH_3 proton of CH_3OH seemed to be affected by solutes. In a *t*-BuOH-H₂O solution, however, no interfering effect on the CH_3 proton of *t*-BuOH was observed, just as in EtOH-H₂O solutions. Thus, the CH_3 protons of CH_3CH_2OH can serve as an internal standard in water-ethanol solution of a constant ethanol content. The effects of solutes on the hydrogen-bonding structure were examined in 25% (v/v) EtOH-H₂O solution, where only the OH signal of H₂O can be observed; however, the signal of EtOH was too broad to be observed. The effects of some alcohols or esters, which are sparingly soluble in lower alcoholic content solution, were examined in 90% (v/v) EtOH-H₂O solutions. The ethanol contents of the *shochu* samples analyzed were in the range of 24.6-25.4% (v/v). The chemical shift values of the *shochu* samples were corrected to 25.0% (v/v) ethanol content, because the value of the OH proton chemical shift varies with ethanol content (12).

Raman Spectra and Analyses of Chemical Components. Raman spectra were recorded using a Horiba-Jobin Yvon LabRam spectrograph HR-800 (Horiba, Ltd., Kyoto, Japan). The spectrometer is equipped with an 1800 g/mm holographic grating. Excitation for the Raman study was provided by a 514.5 nm argon laser. The detector is an ISA air-cooled CCD. The temperature of each sample was regulated at 5 °C by circulating water.

For the total acid measurements, the *shochu* samples were titrated up to pH 8.2 with 0.01 N NaOH aqueous solution; the concentration of total acid was evaluated as that of acetic acid. For *shochu*, the acidity of unity corresponds to 0.001 mol dm⁻³ acetic acid. The quantity of total phenols in *shochu* samples was determined according to the method by Pro (29) using Folin-Ciocalteu's phenol reagent (from Merck) and was evaluated as the amount of tannic acid. The metal ions were analyzed, after sufficient dilutions with water, by a polarized Zeeman atomic absorption spectrophotometer, Hitachi Z-6100 (Hitachi, Ltd., Tokyo, Japan). The anions and organic acids were analyzed by a Dionex ion chromatograph DX-120 (Dionex-Japan, Osaka, Japan), equipped with IonPac AS12A (for the anions) or IonPac ICE-AS6 (for the organic acids) as the analyzing column. The eluents were 0.3 mmol dm⁻³ NaHCO₃ and 0.4 mmol dm⁻³ heptafluorobutyric acid solutions, respectively. The headspace concentrations of flavor components were determined by a Shimadzu gas chromatograph GC-17A (Shimadzu Corp., Kyoto, Japan) after equilibration at 80 °C for 20 min with a 0.22 mm × 25 m PEG-20M (Shimadzu) column. The injector and detector (FID) were both kept at 230 °C. The injection volume was 1 μL and the split ratio 16. The column was operated at 50 °C for 5 min following injection, increased to 200 °C at the rate of 10 °C min⁻¹, and then held at that temperature for 3 min. Amyl alcohol and methyl caproate were used as the internal standards.

RESULTS AND DISCUSSION

1H NMR Chemical Shift Values of *Shochu* Samples. In EtOH-H₂O solution, 1H NMR chemical shifts may reflect the average property or condition of the hydrogen-bonding structure of the three types of hydrogen-bonding interactions: water-ethanol, water-water, and ethanol-ethanol. It is well-known that, with the temperature decrease of 1 °C, the OH proton chemical shift value of H₂O becomes larger (i.e., toward the lower magnetic field) by ~0.01 ppm in pure water (30). The low-field chemical shift corresponds to the strengthening of the hydrogen-bonding structure of water and vice versa. We have already reported that acidic and phenolic components and a few salts (e.g., MgCl₂) could strengthen the hydrogen-bonding structure (23-25).

Table 1. Contents of Acids, Phenols, and Main Inorganic Ions in Different Types of Shochu

type of shochu	content		concn of ions/ppm										
	total acid ^a	total phenol ^b	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cu ²⁺	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻	Fe ²⁺	Mn ²⁺
barley	0.04	0.0	1.5	0.3	0.7	1.0	0.00	7.8	1.2	0.0	4.5	0.00	0.00
barley	0.08	0.0	4.6	0.7	2.2	0.3	0.00	8.6	2.8	0.0	3.1	0.01	0.00
barley	0.06	0.1	5.9	1.1	4.7	0.2	0.00	11.6	0.0	0.0	6.4	0.01	0.00
barley	0.04	0.0	5.6	0.7	0.8	1.2	0.01	9.5	6.9	0.0	6.7	0.01	0.00
barley	0.07	0.0	2.8	1.3	1.8	0.1	0.00	6.9	0.0	0.0	1.2	0.01	0.00
barley (aged)	1.42	60.9	6.6	37.1	2.3	1.1	0.09	13.1	1.7	3.3	17.7	0.12	0.04
barley (aged)	1.10	41.1	0.9	2.4	0.1	0.2	0.03	6.2	0.0	0.0	1.4	0.04	0.00
barley (aged)	0.62	23.7	5.5	2.6	1.1	0.2	0.03	10.3	2.2	0.0	3.1	0.04	0.01
rice	0.62	18.1	3.3	2.2	0.7	0.4	0.45	7.8	3.4	0.0	2.8	0.29	0.02
rice	0.05	1.9	6.2	0.8	1.5	0.8	0.01	7.6	4.4	0.0	1.2	0.00	0.01
rice	0.05	0.1	8.3	0.5	0.0	0.0	0.01	6.9	1.3	0.0	1.9	0.02	0.01
rice	0.08	0.3	1.2	0.5	0.8	0.4	0.07	6.5	2.9	0.0	0.8	0.01	0.01
sweet potato	0.70	2.9	5.5	1.0	0.9	0.2	0.03	10.4	3.4	0.0	4.4	0.03	0.01
sweet potato	0.84	1.4	3.4	0.8	1.0	0.5	0.02	7.4	1.9	0.0	4.7	0.02	0.01
sweet potato	1.04	1.6	0.2	0.2	0.0	0.1	0.03	9.2	1.6	0.0	2.5	0.04	0.01
sweet potato	0.91	2.4	1.7	0.6	0.7	0.9	0.11	7.9	1.7	0.0	4.0	0.03	0.01
sweet potato	0.73	1.6	9.2	1.0	2.7	0.6	0.05	17.1	10.6	0.0	11.6	0.03	0.01
others	0.34	0.0	1.1	0.2	0.1	0.4	0.02	7.5	0.6	0.0	0.8	0.02	0.01
others	0.70	0.5	12.7	0.7	0.6	0.7	0.03	26.4	0.7	0.0	2.1	0.03	0.01
others	0.05	0.3	5.6	0.8	1.4	1.5	0.02	11.0	4.0	0.0	1.9	0.03	0.01
others	0.29	0.0	3.5	1.1	0.8	0.8	0.02	8.5	1.6	0.0	3.1	0.02	0.01
awamori	0.05	1.7	29.6	0.7	0.3	0.6	0.03	21.5	2.5	0.0	13.1	0.02	0.02
awamori	0.30	0.8	7.1	0.5	1.1	7.2	0.02	17.9	0.0	0.0	3.5	0.03	0.02
awamori (aged)	1.26	39.1	33.8	2.8	0.2	0.7	0.06	21.9	3.3	0.0	15.1	0.04	0.02
kou	0.09	0.5	0.3	0.1	0.0	0.1	0.02	7.0	0.0	0.0	0.4	0.02	0.02
kou	0.07	0.0	6.7	1.1	2.3	14.2	0.03	13.6	0.6	0.0	2.6	0.03	0.02
kou	0.40	0.0	1.3	0.2	0.0	0.3	0.04	6.9	0.0	0.9	0.3	0.04	0.02
kou	0.09	1.0	16.0	0.9	0.1	0.1	0.03	14.1	0.0	0.0	2.2	0.02	0.02
kou	0.10	0.3	18.0	0.9	1.4	8.6	0.03	25.2	9.8	0.0	8.6	0.03	0.01

^a The acidity of unity corresponds to 0.001 mol dm⁻³ acetic acid. ^b The quantity of total phenols was evaluated as the amount of tannic acid (ppm).

The contents of total acid, total phenol, and various inorganic ions of shochu samples are shown in **Table 1**. The total acid contents (acidities) of shochu samples were very low: $<1.0 \times 10^{-3}$ mol dm⁻³ (as acetic acid), except for aged samples. According to the ion chromatographic analysis, we found that acetic acid occupied the most part of organic acid contents in every shochu sample. The total acid content of unaged and aged malt whiskey samples (24) were 0.4–1.3 and 4–23, respectively. The total phenol contents in shochu samples were also much smaller than those in aged whiskey samples (200–1000 ppm) (24). The total phenol contents of unaged shochu samples were just in a range from 0 to 3 ppm, although aged shochu samples contained 20–60 ppm.

The maximum molar concentrations of the main inorganic ions were 1.5×10^{-3} mol dm⁻³ (Na⁺), 0.9×10^{-3} mol dm⁻³ (K⁺), 1.9×10^{-4} mol dm⁻³ (Mg²⁺), 3.6×10^{-4} mol dm⁻³ (Ca²⁺), and 0.7×10^{-3} mol dm⁻³ (Cl⁻). As for the concentrations of the main components of shochu, the acidities were $\sim 1/10$ of those of Japanese sake products (25), and the total phenol contents were $<1/10$ of aged whiskey samples (24). The inorganic ions remained at low levels, 10^{-3} – 10^{-4} mol dm⁻³.

Figure 1 shows the changes in the OH proton chemical shifts of the 25% (v/v) EtOH–H₂O solution upon the addition of various solutes at low concentrations. Acetic and vanillic acids of 1×10^{-4} mol dm⁻³ caused a low-field chemical shift. Vanillin also caused a low-field shift at 1×10^{-3} – 1×10^{-2} mol dm⁻³. On the other hand, NaCl did not cause any distinct low-field chemical shift within this concentration range. We have already reported that NaCl (at higher concentrations) causes a high-field chemical shift of OH proton in 20% (v/v) EtOH–H₂O solution; however, in 60% (v/v) EtOH–H₂O solution, this salt of lower concentrations (up to 0.1 mol dm⁻³) can cause

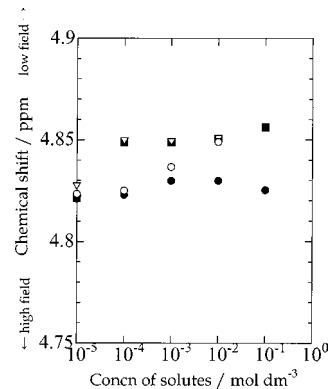


Figure 1. OH proton chemical shifts in 25% (v/v) EtOH–H₂O mixtures containing various solutes: (■) acetic acid; (●) NaCl; (▽) vanillic acid; (○) vanillin.

the low-field chemical shift, that is, the development of hydrogen-bonding structure of water–ethanol (23).

For the first time displayed are the changes of the OH proton signals in the ¹H NMR spectra with the increasing concentration of NaCl (cf. **Figure 2**). The solution of 60% (v/v) EtOH–H₂O gives two OH signals of water and ethanol distinctly (12). In a 60% (v/v) EtOH–H₂O solution containing 1.0×10^{-3} mol dm⁻³ NaCl, both peaks of water and ethanol existed still separately. At 1.0×10^{-2} mol dm⁻³, the peaks of water and ethanol merged into a single peak, indicating that the proton-exchange reaction between water and ethanol molecules was promoted by the dissolved salt. Judging from the narrowed line width in the presence of 1.0×10^{-1} mol dm⁻³ NaCl, the proton exchange seemed to be further promoted. Indeed a further slight sharpening was observed after 1.0×10^{-1} up to 1.0 mol dm⁻³ of NaCl.

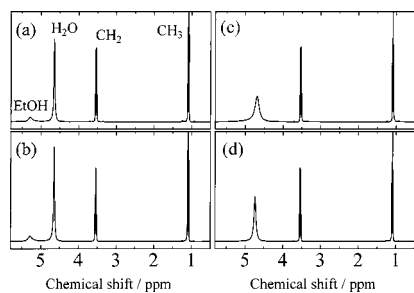


Figure 2. ^1H NMR spectra of 60% (v/v) EtOH–H₂O mixtures containing NaCl of different concentrations. The NaCl concentrations are (a) 0, (b) 1.0×10^{-3} , (c) 1.0×10^{-2} , and (d) 1.0×10^{-1} mol dm⁻³. The peaks on the spectra were assigned to OH of EtOH and H₂O; CH₂ and CH₃ of EtOH refer to the left side of the graph.

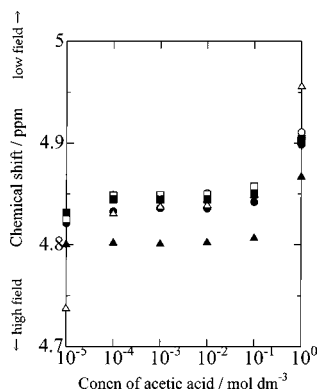


Figure 3. OH proton chemical shifts in EtOH–H₂O mixtures of different ethanol contents with acetic acid concentration. The ethanol contents are (▲) 5.0%, (●) 15%, (■) 20%, (○) 25%, (□) 40%, and (△) 60% (v/v).

Potassium and other alkali metal chlorides gave an effect similar to that by NaCl.

In EtOH–H₂O solutions of various ethanol contents, the effects of acetic acid at low concentrations on the OH proton chemical shifts were examined (Figure 3). Acetic acid at 1×10^{-4} mol dm⁻³ caused a low-field chemical shift in all of the EtOH–H₂O mixtures except for the 5.0% (v/v) EtOH–H₂O mixture. In the 60% (v/v) EtOH–H₂O solution without solute, both of the OH peaks of water and ethanol can be observed distinctly, as mentioned above, and with 1×10^{-5} mol dm⁻³ acetic acid, the two peaks were still observed separately. The OH peak of ethanol is not shown in Figure 3 for simplicity; the ethanol peak was located at a much lower field ($\delta = 5.08$). In the EtOH–H₂O mixture with 1×10^{-4} mol dm⁻³ acetic acid, it was confirmed that the proton exchange between water and ethanol molecules was promoted and the two peaks coalesced to a single peak in the NMR spectrum (23). The lifetime of a hydrogen bond is extremely short (on the order picoseconds); the unification of the two peaks indicates that the proton exchange has become more rapid. With increasing concentration of acetic acid, further low-field chemical shift changes were able to be observed at the same time. Luz et al. (31) examined rate constants of the exchange reactions of ethanol–water mixtures with ^1H NMR spectra, showing that the specific rate of exchange between water and ethanol was proportional to the added acid (or base) concentration. The promotion of proton exchange between water and ethanol molecules accompanied with the strengthened hydrogen bonding has been also observed for benzoic and gallic acids, phenol, or pyrogallol in 60% (v/v) EtOH–H₂O solutions (23).

Figure 4 shows the enlarged OH proton signals in 25% (v/v) EtOH–H₂O with the increased concentration of acetic acid;

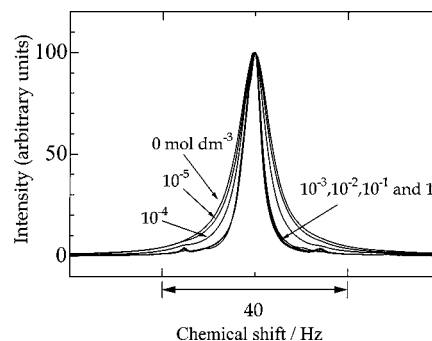


Figure 4. ^1H NMR spectra of OH peaks of 25% (v/v) EtOH–H₂O mixtures containing acetic acid of different concentrations. The acetic acid concentrations are 0, 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 10^{-1} , and 1.0 mol dm⁻³ (in order from the top).

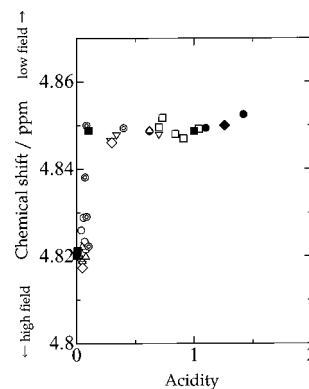


Figure 5. OH proton chemical shifts of shochu as a function of the acidity (in units of 1.0×10^{-3} mol dm⁻³ acetic acid). The types of shochu are (○) barley, (●) barley (aged), (△) rice, (□) sweet potato, (▽) others, (◇) awamori, (◆) awamori (aged), and (⊙) kou. Values for acetic acid solutions of 25% (v/v) EtOH–H₂O are also shown (■).

the line width became narrow and reached a constant value at 1×10^{-3} mol dm⁻³. The low-field chemical shift at 1×10^{-4} mol dm⁻³ acetic acid concentration in the 25% (v/v) EtOH–H₂O mixture (in Figure 1) can be related to the promotion of the proton exchange between water and ethanol molecules.

The OH proton chemical shift values of shochu samples are shown as a function of the acidity (Figure 5). The shochu samples were roughly divided into two groups: the chemical shift values of around 4.82 and 4.85, respectively. For unaged samples, the chemical shift values of the barley types were all around at 4.82. To the contrary, all of the sweet potato types gave the larger chemical shift values (~ 4.85) of the lower field. The shochu samples of the other types, rice, others, and awamori, as well as kou, were divided into two-group values (around 4.82 and 4.85). The chemical shift values of all the aged shochu samples were located at ~ 4.85 . The chemical shift values of shochu samples were all in accordance with the values of acetic acid (displayed by a solid square in the figure) in 25% (v/v) ethanol solution. The acid in shochu would come from fermented wash through distillation; therefore, the procedure of distillation should affect greatly the chemical shift values of shochu. For whiskey maturation in wood casks, the concentrations of acids increase through the direct extraction process from casks or the oxidation of components during maturation in casks (32). The aged shochu samples may gain acidic components likewise during maturation in wood casks.

Line Widths of the Proton NMR Signals of Shochu Samples. In 25% (v/v) EtOH–H₂O mixtures, the effects of various solutes on the line width at half-height of OH proton

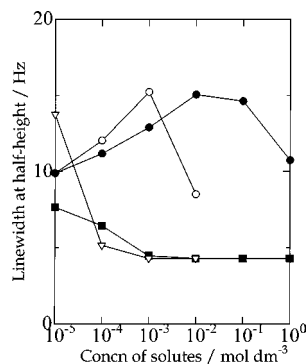


Figure 6. Line widths at half-height of OH proton peaks of ^1H NMR spectra of 25% (v/v) EtOH–H₂O mixtures containing various solutes: (■) acetic acid; (●) NaCl; (▽) vanillic acid; (○) vanillin.

signals of ^1H NMR spectra were examined (Figure 6). As the effect of acetic acid on the width of OH proton peaks in 25% (v/v) EtOH–H₂O solution was already shown in Figure 4, the line width decreased with the acetic acid concentrations and gave the constant values at 1×10^{-3} – 1.0 mol dm^{-3} . The line width at half-height of the OH proton peak of 25% (v/v) EtOH–H₂O solution (without acid) was observed as 8.6 (Hz). Vanillic acid caused the line width of the OH proton peak to increase at $1 \times 10^{-5} \text{ mol dm}^{-3}$ and then to decrease with increasing vanillic acid concentration. Sodium chloride and vanillin had effects similar to that of vanillic acid; that is, the line width of the OH proton peak increased once and then decreased with increasing solute concentration.

Okouchi et al. reported that the line width of ^{17}O NMR signals is dependent on the pH of the solution (33), and they examined the change in the proton-exchange rate in spirits from the relationship between the line width of ^{17}O NMR signals and the pH (4). However, we have already demonstrated that the proton exchange between water and ethanol molecules is promoted by the addition of the neutral buffer (pH 6.86, $0.025 \text{ mol dm}^{-3} \text{ KH}_2\text{PO}_4$ and Na_2HPO_4) to be $1.0 \times 10^{-2} \text{ mol dm}^{-3}$ phosphate ions in 60% (v/v) EtOH–H₂O solution, which should not be subject to the effects of H^+ or OH^- (23). In the present study, we demonstrated that the proton exchange between water and ethanol molecules was promoted and that the peaks of water and ethanol merged into a single peak in ^1H NMR spectra by alkali metal chlorides in 60% (v/v) EtOH–H₂O solution.

The line widths at half-height of the OH proton peaks of shochu samples are plotted against their acidities in Figure 7. The line widths of the OH proton peaks of shochu samples decreased with increasing acidity and became constant at 0.5–1.5 of acidity. In the figure, the line widths at half-height of the OH proton peaks of 25% (v/v) EtOH–H₂O solutions (containing acetic acid) are also shown by solid square symbols. It was found that the line widths of shochu samples were close to those of the 25% (v/v) EtOH–H₂O solutions, excluding some samples of the acidity of ~ 0.1 ; the line widths of these shochu samples were remarkably larger than that of the 25% (v/v) EtOH–H₂O solution of the corresponding acidity ($1.0 \times 10^{-4} \text{ mol dm}^{-3}$ acetic acid). It is true that acetic acid (or a related acid) affects the line widths of the OH proton peaks of shochu samples. However, for the shochu samples, especially those containing very small amount of acids (acidity of ~ 0.1), it is apparent that some chemical components other than acetic acid, such as benzenecarbonic acids, aldehydes, and salts, are concerned with the proton exchange.

Comparison between Raman OH Spectra and ^1H NMR Chemical Shifts. Raman spectroscopy is an effective technique

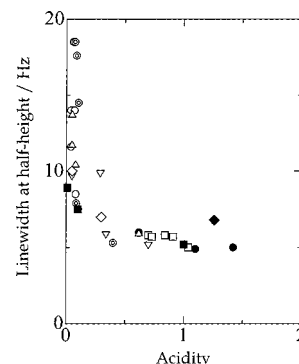


Figure 7. Line widths at half-height of OH proton peaks of ^1H NMR spectra for shochu as a function of the acidity (in units of $1.0 \times 10^{-3} \text{ mol dm}^{-3}$ acetic acid). The types of shochu are (○) barley, (●) barley (aged), (△) rice, (□) sweet potato, (▽) others, (◇) awamori, (◆) awamori (aged), and (⊙) kou. Values for acetic acid solutions of 25% (v/v) EtOH–H₂O are also shown (■).

for examining the hydrogen-bonding structure of water or aqueous solution. Raman spectra are based on the change of molecular polarizability accompanied by vibration or rotation of molecules. The vibrational spectrum of pure liquid water is complicated, and there are various interpretations over assignment of bands (34). At least, however, two large OH stretching bands can be observed in the Raman spectrum of H₂O. There appear to be two classes of OH groups (34): (i) hydrogen-bonded ($\sim 3200 \text{ cm}^{-1}$) and (ii) non-hydrogen-bonded or weakly hydrogen-bonded ($\sim 3400 \text{ cm}^{-1}$) (35, 36). In our previous paper (25), Raman OH stretching spectra have been measured at different temperatures (5, 15, 25, 35, 45, 55, and 65 °C) to investigate the temperature effect on the hydrogen-bonding strength in the 15% (v/v) EtOH–H₂O mixture and Japanese sake samples; the relative intensity of the peak of strongly hydrogen-bonded OH ($\sim 3200 \text{ cm}^{-1}$) to the other peak of non- or weakly hydrogen-bonded OH ($\sim 3400 \text{ cm}^{-1}$) increased with decreasing temperature (that is to say, with strengthened hydrogen bonding). The two OH stretching bands were able to be clearly observed at lower temperatures, such as 5 °C. We paid attention to the ratio of these two peak intensities [$I(3200 \text{ cm}^{-1})/I(3400 \text{ cm}^{-1})$] (at 5 °C) in order to evaluate the degree of hydrogen-bonding strength of water–ethanol. For Japanese sake samples, it has been confirmed, on the basis of the ratio of the Raman intensities as well as the ^1H NMR chemical shifts, that the hydrogen bonding of water–ethanol was strengthened by chemical components such as acids, amino acids, or phenols (25).

In the present study, Raman OH stretching spectra were measured at 5 °C for shochu samples. Figure 8 shows the relationship between the ratios of Raman intensities [$I(3200 \text{ cm}^{-1})/I(3400 \text{ cm}^{-1})$] and ^1H NMR chemical shift values, including the results of Japanese sake samples (data from ref 25). The values of 15 and 25% (v/v) EtOH–H₂O mixtures are also displayed by square symbols in the figure because the ethanol contents of Japanese sake and shochu samples were close to 15 and 25% (v/v), respectively. The ^1H NMR chemical shift values of all the Japanese sake samples were much larger than the value of the 15% (v/v) EtOH–H₂O mixture. Furthermore, the ratios of Raman intensities [$I(3200 \text{ cm}^{-1})/I(3400 \text{ cm}^{-1})$] for almost all Japanese sake samples were larger than that of the 15% (v/v) EtOH–H₂O mixture. Thus, both NMR and Raman methods demonstrated that the hydrogen bonding of water–ethanol in Japanese sake was strengthened.

On the other hand, most of the shochu samples gave larger chemical shift values than the value of the 25% (v/v) EtOH–

Table 2. Contents of Main Flavor Components in Different Types of Shochu

type of shochu	content/ppm							
	acetaldehydes	ethyl acetate	isoamyl acetate	ethyl caproate	ethyl caprylate	propyl alcohol	isobutyl alcohol	isoamyl alcohol
barley	4	5	0.0	0.0	0.0	125	169	508
barley	15	109	6.9	0.7	1.1	162	173	556
barley	4	6	0.0	0.0	0.0	157	204	549
barley	3	49	4.6	0.5	1.6	327	208	646
barley	3	33	3.5	0.3	0.6	197	159	539
barley (aged)	18	14	0.3	0.0	0.3	165	213	596
barley (aged)	16	72	3.6	0.4	1.1	166	201	581
barley (aged)	13	57	2.9	0.3	0.4	134	182	482
rice	25	40	1.5	0.0	0.0	259	220	491
rice	19	85	6.6	0.8	1.2	230	243	530
rice	11	58	3.7	0.3	0.4	203	205	544
rice	22	67	7.6	0.4	0.6	233	263	556
sweet potato	30	104	6.4	0.0	0.9	176	227	492
sweet potato	26	80	3.8	0.0	1.1	98	240	419
sweet potato	19	75	5.5	0.0	1.2	115	276	562
sweet potato	33	90	3.5	0.0	1.3	116	211	458
sweet potato	25	77	4.1	0.0	1.1	121	274	534
others	15	65	2.6	0.3	0.0	190	144	380
others	12	71	2.2	0.3	0.0	175	117	342
others	21	93	7.0	0.7	0.5	250	208	523
others	16	101	7.0	0.4	0.0	90	150	532
awamori	31	56	2.9	0.7	3.2	196	344	598
awamori	52	93	3.2	1.2	1.7	265	266	563
awamori (aged)	46	100	2.6	0.7	3.3	177	366	629
kou	1	3	0.0	0.0	0.0	0	3	2
kou	0	0	0.0	0.0	0.0	0	0	0
kou	1	0	0.0	0.0	0.0	0	0	0
kou	5	14	0.0	0.0	0.0	16	29	91
kou	0	1	0.0	0.0	0.0	0	3	11

H₂O mixture; however, the ratio of the Raman intensities of shochu samples did not increase from the value of the 25% (v/v) EtOH–H₂O mixture. The contents of chemical components were much smaller in the shochu samples, compared with those in the Japanese sake samples; the total acid contents were $\sim 1/100$ or less; the total phenol contents were almost less than 100; furthermore, their amino acid contents were negligibly small. At any rate, the hydrogen bonding of water–ethanol in shochu samples is not strengthened as much as in Japanese sake.

Effects of Volatile Flavor Components on the Hydrogen Bonding of Water–Ethanol. The concentrations of the main flavor components in shochu samples were determined as shown in **Table 2**. Kou shochu is distilled to be of a high ethanol content, such as 94–95% (v/v), just like grain whiskey; therefore, the contents of flavor components in kou samples

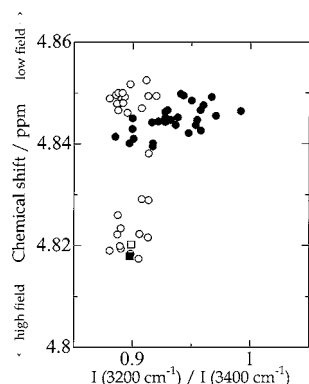


Figure 8. Relationship between the OH proton chemical shift and the ratio of Raman intensities at 5 °C: (○) shochu; (●) Japanese sakes; (■) 15% (v/v) EtOH–H₂O mixture; (□) 25% (v/v) EtOH–H₂O mixture.

were much less than those of shochu samples of other types. However, it was rather difficult to find large differences of flavor components among the shochu samples other than kou.

The effects of higher alcohols as well as esters on the OH proton chemical shifts in 90% (v/v) EtOH–H₂O solution were examined (**Figure 9**). In 90% (v/v) EtOH–H₂O solution, the OH proton peaks of water and ethanol appeared separately. Isoamyl acetate and ethyl caproate caused slightly high-field chemical shifts of both water and ethanol OH proton signals with their increasing concentrations. However, propanol and isoamyl alcohol did not cause any changes in chemical shift values of the two peaks within the solute concentration range.

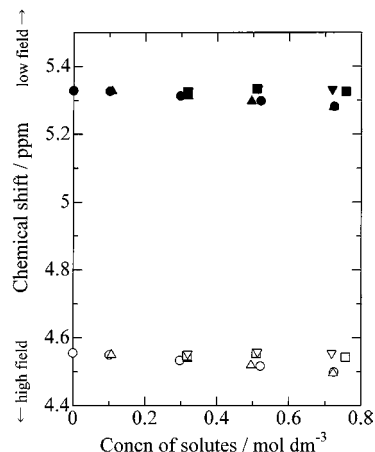


Figure 9. OH proton chemical shifts of H₂O (open symbols) and EtOH (solid symbols) in 90% (v/v) EtOH–H₂O mixtures containing esters and higher alcohols: (○) ethyl caproate; (△) isoamyl acetate; (▽) propyl alcohol; (□) isoamyl alcohol.

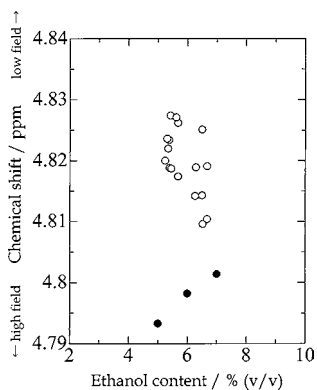


Figure 10. OH proton chemical shift of cocktails of low ethanol content (open symbols) and EtOH–H₂O mixtures (solid symbols).

Thus, the higher alcohols and esters caused no development in hydrogen bonding of ethanol and water solution.

It was found that the hydrogen-bonding structure of water–ethanol in shochu (white liquor) was slightly affected by the chemical components, at a much lower level than that in aged whiskey (brown liquor) and Japanese sake (brewage). In some shochu samples, the hydrogen bonding of water–ethanol was strengthened by the small amounts of acids, to an extent that the proton exchange between water and ethanol molecules could be promoted. However, in the other shochu samples, the OH proton chemical shift values as well as the ratios of Raman intensities were so close to those of the mere 25% (v/v) EtOH–H₂O solution that the additional development of the hydrogen-bonding structure could not be expected at all.

Figure 10 shows the OH proton chemical shift values in carbonated fruit cocktail drinks. The samples were ultrasonically degassed before the ¹H NMR chemical shift values were measured. Basically, fruit cocktails are made by mixing various fruit juices and neutral spirits. Some types of shochu are used also as a material of mixed liquors or cocktails because of their high purity. It was found, on the basis of ¹H NMR chemical shift values, that the hydrogen bonding of water–ethanol in the fruit cocktails was strengthened, compared with that of the EtOH–H₂O mixture of the same ethanol content. The acidic and (poly)phenolic substances from fruit juices can cause a development of the hydrogen-bonding structure of the diluted neutral spirit immediately.

In the manufacturing process of gin, for instance, the spirit and botanicals are charged into a pot still, so the volatile components of the botanical constituents are recognizably present in the product (37). On the other hand, for a type of vodka, sodium and calcium salts may contribute to the mellowness (37). We believe that organic acids or (poly)phenols (proton donors) and conjugate base anions of weak acids (proton acceptors) are the main components to reduce the organoleptical stimulation of alcoholic beverages: the secondary effects for the mellowness may be brought about by other substances, such as flavor and salty components. Sugar components are essential to moderate the sour and astringent tastes by acids and (poly)phenols.

So far, no one has demonstrated a concrete relationship scientifically between the water–ethanol structure and the reduction of alcoholic stimulation for the mucous membranes of human beings or animals. Now that the difference of the hydrogen-bonding structure between alcoholic beverages and the mere water–ethanol mixtures has been revealed, we are able to consider and determine without difficulty what would cause the water–ethanol structural change.

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