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## Solute Effects on the Interaction between Water and Ethanol in Aged Whiskey

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The hydrogen-bonding structure of water–ethanol in whiskey was examined on the basis of  $^1\text{H}$  NMR chemical shifts of the OH of water and ethanol. Phenolic acids and aldehydes (gallic, vanillic, and syringic acids; vanillin and syringaldehyde) exhibited their structure-making effects regardless of the presence or absence of 0.1 or 0.2 mol dm $^{-3}$  acetic acid. The OH-proton chemical shifts were measured for 32 malt whiskey samples of a distillery, aged for 0–23 years in five different types of casks. The OH-proton chemical shift values of the whiskeys shifted toward the lower field in proportion to their contents of total phenols. It can be concluded that the strength of the hydrogen bonding in aged whiskeys is directly predominated by acidic and phenolic components gained in oak wood casks and not dependent on just the aging time.

**KEYWORDS:** Chemical shift in  $^1\text{H}$  NMR; hydrogen bonding; water structure; ethanol; whiskey; whisky; aging; mellowness; gallic acid; anthocyanin; vanillin; lactone

### INTRODUCTION

Distilled spirits such as whiskey and brandy are stored in oak wood casks for more than several years in their usual manufacturing process. Through this long maturation, the stimulation and odor peculiar to ethanol in spirits are reduced; consequently, their tastes are altered to be favorable for alcoholic beverages. The reduction of stimulation in the taste of aged spirits might be related to the change of the structure of water and ethanol molecules (1). So far, however, no one has demonstrated the relationship scientifically between the water–ethanol structure and the change of taste. Several studies have been reported upon the water–ethanol structure change in distilled spirits with different analytical methods. Akaoshi and Ohkuma (2) have examined the chemical shifts and half-bandwidths of  $^1\text{H}$  NMR spectra, and others (3–6) have analyzed the half-bandwidths of  $^1\text{H}$  or  $^{17}\text{O}$  NMR spectra. Furusawa et al. (7) examined the ethanol–water cluster by means of mass spectroscopy, Koga and Yoshizumi (8, 9) pointed out the role of components in aged whiskey based on the data by differential scanning calorimetry (DSC).

Despite their extensive efforts, the cause of the water–ethanol structure change in aged spirits has not been clarified sufficiently. Some DSC thermograms may have demonstrated that nonvolatile fractions of the matured whiskey cause the stronger interaction between water and ethanol molecules (1, 9). Aka-

hoshi and Ohkuma (2) noticed a slight effect of oak wood extracts on the half-bandwidth of the  $^1\text{H}$  NMR spectra of the OH-proton; however, they noted that the effect was very small, compared with all the changes throughout aging. Furusawa et al. (7) suggested that some volatile and nonvolatile compounds could assist the formation of ethanol–water clusters in whiskey. To consider the water–ethanol structural change related to the reduction of ethanol stimulation, it is essential to find the cause of the water–ethanol structural change. Conner et al. (10) noted that organic acids increased the solubility of distillate compounds in aged spirits during maturation.

We have investigated the effects of various components contained in alcoholic beverages on the hydrogen-bonding structure of water–ethanol, analyzing the chemical shift of  $^1\text{H}$  NMR, to find out that acidic and phenolic components and a few salts (e.g.  $\text{MgCl}_2$ ) could strengthen the hydrogen-bonding structure (11). Indeed whiskey contains many components, affecting the senses of sourness, sweetness, bitterness, and saltiness. So far, however, it has never been proposed that any specific component in whiskey is related directly to the reduction of alcoholic stimulation. Many kinds of whiskey components, such as acids, phenols, or aldehydes, are extracted and subjected to conversion in (well-charred) wood casks during maturation. The amounts of extracts from wood casks in matured whiskeys are seriously dependent on the cask conditions, that is, the cask type, the cask history, and prior treatment of the cask as well as warehouse conditions (12–14). The concentrations of

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components should vary with casks in which whiskies have been matured, even if their aging periods are the same.

In the present paper, the OH-proton NMR chemical shifts were measured for many malt whiskies, which had been aged in oak wood casks of different types, that is, used, remade, recharred, new, and sherry types. The factors predominating the change of the hydrogen-bonding structure have been investigated. We focused on the relationship between the hydrogen-bonding structure of water–ethanol and acidic and phenolic compounds, lactones, sugars, or esters.

## MATERIALS AND METHODS

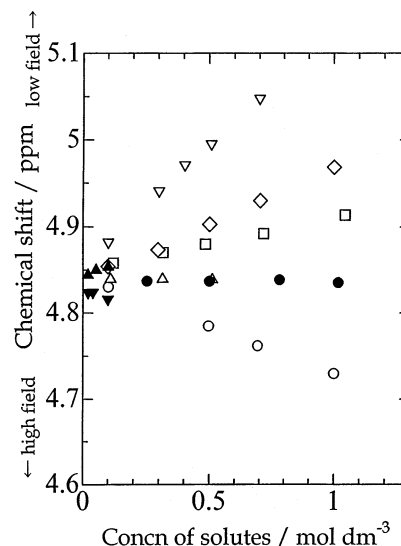
**1. Chemicals.** Commercially obtained acids of analytical grade were used without further purification: formic (98.0%, Wako), oxalic (99.5%, Wako), acetic (99.0%, Nacalai), phenylacetic (Wako), L-(+)-lactic (98%, Sigma), succinic (99%, Aldrich), malic (99%, Aldrich), L-(+)-tartaric (99.5+%, Wako), citric (98+%, Wako), L-(+)-ascorbic (99.5%, Wako), gallic (97%, Aldrich), vanillic (98%, TCI), and syringic acids (97%, TCI). Other chemicals were also of analytical grade: (+)-catechin (98%, Sigma), caffeine (98.5%, Wako), acetaldehyde (99%, Aldrich), benzaldehyde (98.0%, Wako), *p*-hydroxybenzaldehyde (98.0%, Wako), vanillin (98.0+%, Wako), syringaldehyde (98%, TCI), delphinidin chloride (Extrasynthese), D-(−)-pantolactone (Sigma),  $\gamma$ -octanolactone (TCI), C<sub>2</sub>H<sub>5</sub>OH (Wako, GR grade), sodium acetate (98.5%, Wako), and tannic acid (ACS reagent, Aldrich). Other metal salts and chemicals of analytical grade were used as received. Water was purified by means of a MilliQ System (Millipore Corporation).

**2. Whiskey Samples.** Malt whiskey samples were brewed, distilled, and aged in a distillery (Nikka Whisky Sendai distillery, Japan). The samples aged in five kinds of casks are as follows: new (first fill), used (refill), recharred, remade (parts of a used cask are replaced), and sherry casks. Whiskey samples of different aging periods were taken out from different casks at the same time, so naturally the malt whiskies before aging are different from each other. It is true that there should be different conditions in the malt whiskey manufacturing processes, such as fermentation, distillation, and aging; however, they are all of “single casks” and not the commercial products blended of different origins.

**3. <sup>1</sup>H NMR Chemical Shifts.** The <sup>1</sup>H NMR chemical shifts of the hydroxyl groups in EtOH–H<sub>2</sub>O solutions containing various compounds or whiskey samples were measured with a JEOL JNM-LA 400 NMR spectrometer (JEOL, Ltd., Tokyo Japan) at 25 ± 0.1 °C in Wilmad coaxial tubes (11). D<sub>2</sub>O (99.9% D, Aldrich) in the inside tube served as the locking solvent. The center peak of the triplet peak of the methyl group was utilized as the standard (CH<sub>3</sub>, 1.164 vs TMS) (11, 15). In 20% (v/v) EtOH–H<sub>2</sub>O solution, the OH peaks of water and ethanol are observed as a single peak; that is, the OH peaks cannot be given separately, where the water structure is regarded to be most strengthened in all ranges of ethanol contents. The effects of solutes on the hydrogen-bonding structure were examined by analyzing the chemical shift of <sup>1</sup>H NMR mainly in 20% (v/v) EtOH–H<sub>2</sub>O solution. In 60% (v/v) EtOH–H<sub>2</sub>O solution, on the other hand, two peaks appear separately; the proton chemical shift of the water is observed at higher magnetic field than that in 20% (v/v) EtOH–H<sub>2</sub>O solution (16). The effects of some acids or aldehydes which are sparingly soluble in lower alcoholic content solution were examined in 60% (v/v) EtOH–H<sub>2</sub>O solutions. The chemical shift values ( $\Delta\delta$ ) per 1.0 mol dm<sup>−3</sup> solutes were estimated after confirming the linearity between chemical shifts and their concentrations.

The chemical shift values were represented for 32 whiskey samples of which ethanol contents were within 60–67% (v/v) because the chemical shift values of EtOH–H<sub>2</sub>O samples were verified not to be influenced by the ethanol-content changes within this range. Other analyses were carried with all the 39 malt whiskey samples, of which ethanol contents were within 51–67% (v/v). The measurements were also done for six unaged malt whiskey samples.

**4. Analyses of Whiskey Components.** For the total acid measurements, the whiskey samples were titrated up to pH 8.2 with 0.01 N NaOH aqueous solution; the concentration of total acid was evaluated



**Figure 1.** OH-proton chemical shifts in 20% (v/v) EtOH–H<sub>2</sub>O mixtures containing various compounds: (○) NaNO<sub>3</sub>; (△) NaHCO<sub>3</sub>; (□) formic acid; (▽) oxalic acid; (◇) L-(+)-ascorbic acid; (●) sodium acetate; (▲) (+)-catechin; (▼) caffeine.

as that of acetic acid. For whiskey, the acidity of unity corresponds to 0.001 mol dm<sup>−3</sup> acetic acid. The quantity of total phenols in whiskey samples was determined according to the method by Pro (17) using a Folin-Ciocalteu's phenol reagent from Merck and was estimated as the amount of tannic acid. The glucose concentration in aged whiskies was determined colorimetrically (18) using a glucose determination kit from Shino-Test Corporation. The metallic components in whiskies were analyzed (19), after good dilutions with water, with a polarized Zeeman atomic absorption spectrophotometer Hitachi Z-6100 (Hitachi, Ltd., Tokyo, Japan).

## RESULTS AND DISCUSSION

**1. Effects of Solute on the Water–Ethanol Structure. 1.1. <sup>1</sup>H NMR Chemical Shifts and the Hydrogen-Bonding Structure.** It is well-known that, with a temperature decrease of 1 °C, the OH-proton chemical shift value of H<sub>2</sub>O becomes larger by about 0.01 ppm (toward the lower magnetic field) in pure water (20). The chemical shift toward lower field corresponds to the strengthening of the hydrogen-bonding structure of water, and vice versa. In the <sup>1</sup>H NMR study of EtOH–H<sub>2</sub>O solution, we have found that almost all alkali metal and alkaline earth metal salts (from strong acids) cause high-field chemical shifts; on the other hand, not only acids and phenols (hydrogen-bonding donors) but also conjugate-base anions (hydrogen-bonding acceptors) from weak acids can cause low-field chemical shifts. The degree of the effect is dependent on the acid strength (pK<sub>a</sub>) and the number of carboxyl (−COOH) and hydroxyl (−OH) functions in an acid molecule. We would like to note that undissociated acid molecules (HA) as well as the proton (H<sup>+</sup>) cause the effect of strengthening the structure of water or water–ethanol (11). It is true that the <sup>1</sup>H NMR chemical shift values vary with the concentration of H<sup>+</sup> (or pH), however, one should notice that undissociated acidic and phenolic components rather than H<sup>+</sup> itself cause the main contribution to the chemical shift of alcoholic beverages.

**Figure 1** shows the changes in the OH-proton chemical shifts of the 20% (v/v) EtOH–H<sub>2</sub>O solution upon the addition of solutes of various concentrations. Formic and oxalic acids caused the low-field chemical shifts (cf. **Table 1**); the chemical shift values were well accordant with the pK<sub>a</sub> values and the number of carboxyl groups of the acids, though these acids have no

**Table 1.** OH-Proton Chemical Shifts in 20% (v/v) EtOH–H<sub>2</sub>O Solutions Containing Acidic and Phenolic Compounds

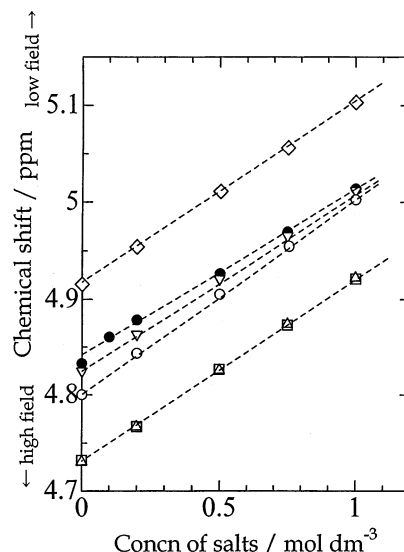
compound	pK <sub>a</sub> in H <sub>2</sub> O <sup>a</sup>	change in chemical shift <sup>b</sup> (ppm)
formic acid	3.75	0.060
acetic acid	4.75	0.064 <sup>c</sup>
phenylacetic acid	4.28	0.056
oxalic acid	1.23, 4.19	0.276
L-(+)-ascorbic acid	4.10, (11.79)	0.131
benzoic acid	4.2	0.122 <sup>d,e</sup>
gallic acid	4.46	0.363 <sup>d</sup>
chlorogenic acid		0.460 <sup>d</sup>
tannic acid		ca. 2.0 <sup>d</sup>
pyrogallol	9.24	0.054 <sup>d</sup>
(+)-catechin		0.114
(-)-epigallocatechin gallate		0.480 <sup>d,e</sup>
delphinidin chloride		0.352

<sup>a</sup> Cited mainly from ref 21. <sup>b</sup>  $\Delta\delta$  per 1.0 mol dm<sup>-3</sup> acids or phenols. The values were estimated from the data of lower concentrations below 1.0 mol dm<sup>-3</sup>. <sup>c</sup> A larger value of 0.071 had been evaluated from a very wide concentration range of 0.5–3.0 mol dm<sup>-3</sup>; cf. ref 11. <sup>d</sup> Reference 11. <sup>e</sup> The values were estimated below 0.05 mol dm<sup>-3</sup> because of their low solubilities.

alkyl group in the molecules. The effect of phenylacetic acid on the OH-proton chemical shifts in 20% (v/v) EtOH–H<sub>2</sub>O solution turned out to be close to that of acetic acid ( $\Delta\delta = 0.056$  and  $0.064$  per 1.0 mol dm<sup>-3</sup>, respectively). The effects of these acids on the OH-proton chemical shift seem not to be so much dependent on the presence or absence of a nonpolar group, although nonpolar groups in solute molecules have been regarded to affect the hydrogen bonding of the solvent water (15). L-(+)-Ascorbic acid, contained often in various drinks as Vitamin C, gave the low-field chemical shift  $\Delta\delta = 0.131$ ; and (+)-catechin (abundant in wine and tea) also gave the low-field chemical shift  $\Delta\delta = 0.114$  (cf., 0.480 of (-)-epigallocatechin gallate possessing an additional gallic acid part in the molecule). However, the low-field chemical shift was not given by caffeine. The apparent effects of sodium salts (Na<sup>+</sup>A<sup>-</sup>) were dependent on the anions coming from either strong or weak acids.

**1.2. Effectiveness of Carboxylic Acids in the Presence of Salts.** Before maturation in wood casks, freshly distilled whiskies contain organic acids from fermented wash through distillation (19), and the concentrations of acids increase through the direct extraction process from casks or oxidation of components during maturation in casks (14). Aged whiskies contain also inorganic salts; some of them increase through elution from wood components of casks (19). In the present study, the concentrations of metal cations (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup>) were determined for all whiskey samples to find that some samples contained K<sup>+</sup> of more than 100 ppm (vide infra).

**Figure 2** shows the OH-proton chemical shifts in 20% (v/v) EtOH–H<sub>2</sub>O solutions with the malic acid concentration in the presence of 1.0 (or 0.5) mol dm<sup>-3</sup> different salts. Malic acid (in the absence of a salt) caused low-field shifts in proportion to its concentration. The salts of the structure-breaking effect (NaCl, KCl, CaCl<sub>2</sub>) gave the high-field shift at 0 mol dm<sup>-3</sup> malic acid while MgCl<sub>2</sub> of the structure-making effect gave the low-field shift. In **Figure 2**, the slope with a salt was definitely steeper than that without salt. The values of chemical shift per 1.0 mol dm<sup>-3</sup> malic acid in the EtOH–H<sub>2</sub>O solution containing the salts, evaluated from the line slopes, were shown in **Table 2**; the effect of Ca<sup>2+</sup> was larger than that of Mg<sup>2+</sup>, Na<sup>+</sup>, or K<sup>+</sup>. The results for other acids in the presence of 1.0 mol dm<sup>-3</sup> NaCl are also listed. We can find that the chemical shift change (the line slope) caused by an acid containing NaCl is larger than that without the salt: the ratios of chemical shift changes

**Figure 2.** Changes of OH-proton chemical shifts in 20% (v/v) EtOH–H<sub>2</sub>O mixtures with malic acid concentration in the presence of 1.0 mol dm<sup>-3</sup> different salts (0.5 and 1.0 mol dm<sup>-3</sup> only for CaCl<sub>2</sub>): (●) no salt; (□) NaCl; (Δ) KCl; (◇) MgCl<sub>2</sub>; (▽) 0.5 mol dm<sup>-3</sup> CaCl<sub>2</sub>; (○) CaCl<sub>2</sub>.**Table 2.** Effect of Acids (1.0 mol dm<sup>-3</sup>) on the OH-Proton Chemical Shifts in 20% (v/v) EtOH–H<sub>2</sub>O Mixtures in the Absence or Presence of 1.0 mol dm<sup>-3</sup> NaCl, KCl, MgCl<sub>2</sub>, and CaCl<sub>2</sub>

component	change in chemical shift <sup>a</sup> (ppm)					ratio <sup>b</sup>
	no salt	NaCl	KCl	MgCl <sub>2</sub>	CaCl <sub>2</sub>	
acetic acid	0.064	0.070				1.09
lactic acid	0.088	0.096				1.09
succinic acid	0.136	0.150				1.10
malic acid	0.170	0.190	0.192	0.187	0.202	1.12
tartaric acid	0.195	0.226				1.16
citric acid	0.253	0.280				1.11

<sup>a</sup>  $\Delta\delta$  (without salt) and  $\Delta\delta'$  (with salt) per 1.0 mol dm<sup>-3</sup> acid, evaluated from the line slope. <sup>b</sup> The ratio of chemical shift change in the presence of NaCl to that without salt.

(NaCl/no salts) were about 1.10 for all acids, although tartaric acid (possessing three carboxyl functions and one OH function) gave a slightly larger ratio (1.16).

When an acid and a salt coexist in EtOH–H<sub>2</sub>O solution, the acid and the metal cation may interact mutually. The interaction between carboxyl groups of acids and the metal cations in the solution could release the proton as follows.

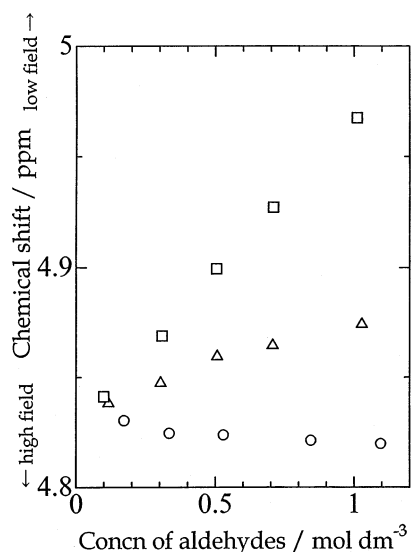


The produced proton can cause a remarkable low-field chemical shift ( $\Delta\delta(\text{H}^+) = 0.424$  per 1.0 mol dm<sup>-3</sup> H<sup>+</sup>) in 20% (v/v) EtOH–H<sub>2</sub>O solution (11). The hydrating power toward the cations in aqueous solution becomes stronger as the radius of the ions (per charge) decreases (22). The solvation toward metal cations should reduce the rather strong interaction (23) between carboxylate ions and the metal cations.

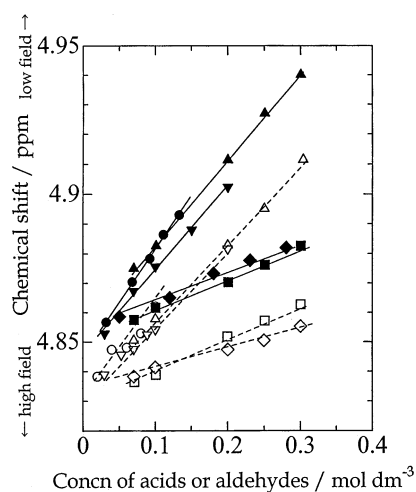
**1.3. Effectiveness of Benzenecarboxylic Acids and Aldehydes in the Presence of Acetic Acid.** Acetaldehyde also exists before maturation, and the amount of it increases through the oxidation of ethanol during maturation (14). Aromatic aldehydes, such as syringaldehyde and vanillin, are derived from oak wood lignins, and their concentrations in spirits increase during maturation in casks (13, 14, 24).

**Figure 3** shows the effects of aldehydes on the OH-proton chemical shifts in 60% (v/v) EtOH–H<sub>2</sub>O solutions. Acetalde-





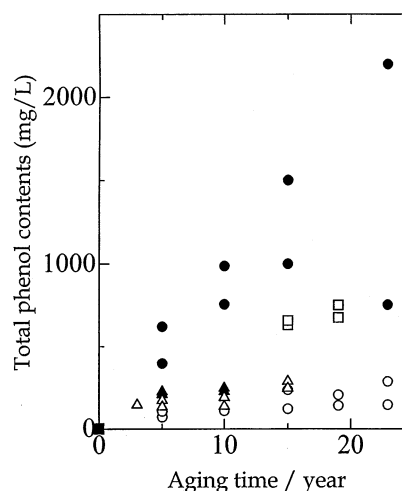
**Figure 3.** OH-proton chemical shifts in 60% (v/v) EtOH-H<sub>2</sub>O mixtures containing various aldehydes: (○) acetaldehyde; (△) benzaldehyde; (□) *p*-hydroxybenzaldehyde.



**Figure 4.** Changes of OH-proton chemical shifts in 60% (v/v) EtOH-H<sub>2</sub>O mixtures with benzenecarboxylic acid and aldehyde concentrations in the absence (open symbols) and the presence (solid symbols) of 0.2 mol dm<sup>-3</sup> acetic acid (0.1 mol dm<sup>-3</sup> only for gallic acid): (○) gallic acid; (△) vanillic acid; (▽) syringic acid; (□) vanillin; (◇) syringaldehyde.

hyde gave no low-field chemical shift, while benzaldehyde and *p*-hydroxybenzaldehyde showed clearly low-field shifts: aldehydes possessing a benzene ring are indeed effective in strengthening the hydrogen-bonding structure of water-ethanol. Furthermore, the hydroxyl group in *p*-hydroxybenzaldehyde caused an enhanced structure-making effect. Such enhanced effects caused by possessing the benzene or the phenol group have been recognized for carboxylic acids (11).

The effectiveness of benzenecarboxylic acids or aldehydes, the important components in the matured whiskey, was examined in the presence of acetic acid in 60% (v/v) EtOH-H<sub>2</sub>O solution (Figure 4). Even if these acids or aldehydes coexisted with 0.1 or 0.2 mol dm<sup>-3</sup> acetic acid, they still exhibited their effects of strengthening the water-ethanol structure as largely as those without acetic acid; for example, for vanillic acid,  $\Delta\delta' = 0.283$  and  $\Delta\delta = 0.261$  (per 1.0 mol dm<sup>-3</sup>) in the presence and the absence of 0.2 mol dm<sup>-3</sup> acetic acid, respectively. The effect of these acids or aldehydes turned out to be almost independent of the coexistence of acetic acid.

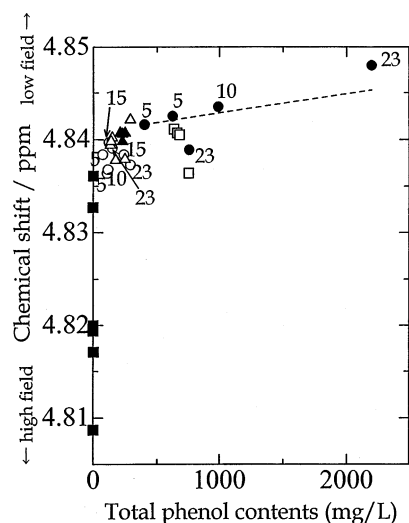


**Figure 5.** Change in total phenol contents of whiskeys matured in casks of different kinds with aging time: (○) used; (△) recharred; (□) new; (●) sherry; (▲) remade; (■) unaged.

**2. Structural Change of Water-Ethanol in Whiskey. 2.1. Acidic and Phenolic Compounds in Whiskey.** Various kinds of compounds come from wood casks, such as gallic acid or vanillin, and their amounts increase with aging time. The acetic acid concentration also increases through elution from the cask and oxidation of ethanol, in addition to the small amount of the acid before maturation. Pathways for the origin of lignin-related compounds in matured spirits have been proposed (1). The increase of the color of whiskeys has been found to be related with the contents of congeners such as esters, aldehydes, solids, or organic acids (14, 25). The effects of wood type (botanical) or wood treatment have been summarized (12). In the present study, we were able to demonstrate an absolute linearity between the color intensity and the total phenol contents in aged whiskeys.

Figure 5 shows the changes of the total phenol contents in whiskeys with their aging time. For the whiskeys aged in sherry casks, their total phenol contents increased with aging time, and the amounts were much higher than those in other kinds of casks. The whiskeys aged for 15–19 years in new casks had fairly high total phenol contents. The total phenol contents in recharred or remade casks seemed to increase with aging time; however, those in used casks remained at very low levels. The effects of extraction from casks were significantly dependent on the kind of the cask. Judging from the increase in total phenol contents, the extractive effect from casks increases as follows: used < recharred < remade < new < sherry. We verified that the total phenol contents in whiskey are affected significantly by the kind of the cask, even if the aging time is the same. We should note that an excellent correlation was observed between the contents of total phenols and total acids (or the acidity) in the aged whiskeys.

**2.2. Chemical Shift Values of Unaged Whiskeys.** The OH-proton NMR chemical shift values of the whiskey samples were shown against the total phenol contents (Figure 6). The chemical shift values of the unaged whiskeys (ethanol contents of 64.6–65.6% (v/v)) were located around 4.81–4.835. In 66% (v/v) EtOH-H<sub>2</sub>O solution, the proton chemical shift of the water is observed at much higher field ( $\delta = 4.701$  ppm), and its ethanol OH signal is located at much lower field ( $\delta = 5.36$  ppm). The total acid contents of the unaged whiskeys were from 30 to 90 mg/L, and their total phenol contents were negligibly small (within 0–5 mg/L). However, the small amount of acids in unaged whiskey samples was effective enough to merge the



**Figure 6.** OH-proton chemical shifts of whiskeys matured in casks of different kinds as a function of their concentration of total phenols: (○) used; (△) recharred; (□) new; (●) sherry; (▲) remade; (■) unaged. The dotted line shows the effects of gallic acid; see the text.

two peaks of water and ethanol into a single peak. The total acid content of 60 mg/L corresponds to  $1.0 \times 10^{-3}$  mol dm<sup>-3</sup> acetic acid. It has already been demonstrated that the OH peaks of water and ethanol are coalesced to a single peak by the addition of as low as  $1.0 \times 10^{-4}$  mol dm<sup>-3</sup> acetic acid in 60% (v/v) EtOH–H<sub>2</sub>O solution (11). Accordingly, it must be quite natural to observe the fact that, with the small amount of acids in the unaged whiskeys, not only the proton exchange between water and ethanol has been promoted but also the hydrogen bonding structure has been slightly strengthened even before maturation in casks. Furusawa et al. (7) have proposed that the unaged whiskeys induce ethanol–water clusters to a higher extent than that for the simple EtOH–H<sub>2</sub>O mixture. Incidentally, we have observed that the two peaks of water and ethanol are merged into a single peak by the addition of the neutral buffer (pH 6.86, 0.025 mol dm<sup>-3</sup> KH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>) to be  $1.0 \times 10^{-2}$  mol dm<sup>-3</sup> phosphate ions in 60% (v/v) EtOH–H<sub>2</sub>O solution, which should not be subjected to the effects of H<sup>+</sup> or OH<sup>-</sup> (11).

**2.3. Aged Whiskeys.** The total phenol contents of some of aged whiskeys were 200–300 mg/L, with their chemical shift values being raised to around 4.835–4.84. With increasing content of total phenols in whiskeys, the chemical shift values increased from 4.84 up to 4.85. The whiskeys aged in sherry, new, or remade casks had gained lots of phenolic (and acidic) compounds, and then those compounds would cause the development in the hydrogen-bonding structure of whiskeys. To the contrary, the whiskeys aged in less extractive casks (such as used casks) contained only small amounts of phenolic (and acidic) compounds, resulting in less development of hydrogen-bonding structure.

The change of the chemical shift value caused by 1.0 mol dm<sup>-3</sup> gallic acid has been evaluated to be  $\Delta\delta = 0.348$  in 60% (v/v) EtOH–H<sub>2</sub>O solution (11). The corresponding effect of gallic acid is displayed with the dotted line in **Figure 6**. (Note the concentration 1000 mg/L corresponds to  $5.9 \times 10^{-3}$  mol dm<sup>-3</sup> for gallic acid.) The aging years were displayed for whiskey samples of sherry casks and used casks on the symbols in **Figure 6**. For the whiskeys aged in sherry casks, their total phenol contents increased with the aging time (5, 10, and 23 years); at the same time, the chemical shift values increased

(excluding an exceptional sample) in proportion to the phenol contents. In contrast, the total phenol contents in used casks did not increase so much even after long aging time, up to 23 years: the chemical shift values remained below 4.84. In other words, as for the whiskeys aged in the used casks, the hydrogen-bonding structure of water–ethanol had not been so developed even after long maturation time, such as 15 or 23 years.

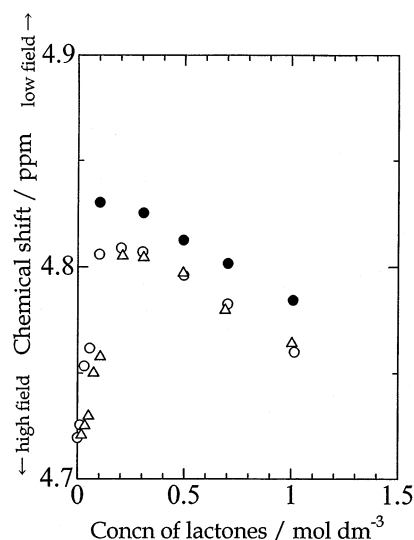
It has been reported that the K<sup>+</sup> concentration increases in whiskeys with aging time and is the highest of all metal cations (19). We were also able to confirm that the K<sup>+</sup> concentration is the highest and is properly correlated to the total phenol contents of aged whiskeys. The K<sup>+</sup> concentrations in whiskey samples matured in sherry casks were 106 ppm (5 years), 108 ppm (5 years), 45 ppm (23 years), 148 ppm (10 years), and 192 ppm (23 years), of the order (from the left side) displayed in **Figure 6**. The 23-year-aged whiskey sample, of the exceptionally small chemical shift value, had the smallest K<sup>+</sup> concentration out of five whiskey samples aged in sherry casks. The whiskey samples aged in used casks gained the metal ion of low levels (<30 ppm) even after 23 years. Note that the concentrations of K<sup>+</sup> in the drinking waters of Japan are usually much less than 10 ppm.

We can conclude that the change of hydrogen-bonding structure of whiskey during maturation in wood casks is due to the direct effect of chemical components from casks (mainly acidic and phenolic compounds or aldehydes, such as vanillin). A model for the tight association among H<sub>2</sub>O and EtOH, assisted by carboxylic acid (RCOOH), the conjugate-base anion (RCOO<sup>-</sup>), and gallic acid [(OH)<sub>3</sub>C<sub>6</sub>H<sub>2</sub>COOH], has been proposed in the previous paper (11).

**2.4. Effects of Anthocyanins and Aldehydes.** The total phenol contents of whiskeys aged in sherry casks were found to be much higher than those in any other casks, as mentioned above. The sherry (a kind of wine, prepared by a special procedure) is supposed to contain many phenolic compounds from the grapes. Delphinidin is one of the several types of anthocyanins in grapes. The OH-proton chemical shift caused by the delphinidin (cation) in 20% (v/v) EtOH–H<sub>2</sub>O solution was evaluated to be  $\Delta\delta = 0.400$ ,  $\Delta\delta(\text{delphinidin cation}) + \Delta\delta(\text{Cl}^-) = 0.352$ , where  $\Delta\delta(\text{Cl}^-) = -0.048$  (11); delphinidin has a large effect of strengthening the hydrogen-bonding structure of water–ethanol; cf.  $\Delta\delta = 0.363$  of gallic acid or  $\sim 2.0$  of tannic acid (the ester of a glucose with many gallic acids, with molecular weight 1701.23) in solutions with the same ethanol content (11). An anthocyanin, one of the total phenolic components, could cause the development of the hydrogen-bonding structure of whiskeys aged in sherry casks. Incidentally, the influence of wine polyphenols on the volatility of aroma substances has been examined (26, 27).

The oxidation processes of several compounds in whiskey are regarded to be important reactions for whiskey maturation. Some aldehydes in whiskey are oxidized into acids during the maturation (14). The chemical shift values ( $\Delta\delta$ ) increased much with the oxidation from aldehydes to acids: acetaldehyde ( $-0.009$ )  $\rightarrow$  acetic acid (0.118); syringaldehyde (0.070)  $\rightarrow$  syringic acid (0.250); vanillin (0.114)  $\rightarrow$  vanillic acid (0.261) in 60% (v/v) EtOH–H<sub>2</sub>O solution (11). Accordingly, the hydrogen-bonding structure of whiskey is likely to be strengthened through the oxidation reactions during maturation.

**2.5. Effects of Lactones, Sugars, and Esters.** Lactones are believed to contribute to the flavor characteristic of whiskey (28, 29). Some of them are derived from wood components of a cask (30); others are produced in fermented wash by the work of brewers' yeasts and lactic acid bacteria (31).



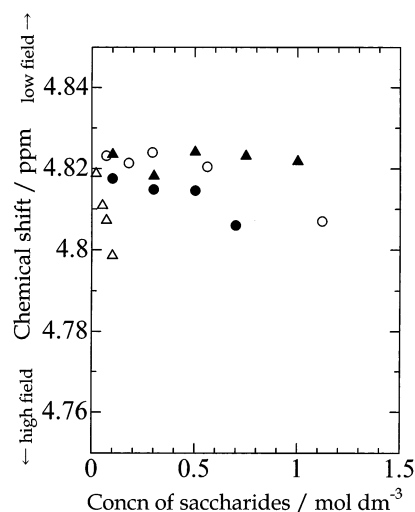
**Figure 7.** OH-proton chemical shifts in 20% (v/v) (solid symbols) and 60% (v/v) (open symbols) EtOH-H<sub>2</sub>O mixtures containing lactones: (○) D-(-)-pantolactone; (Δ) γ-octanolactone.

**Figure 7** shows the effects of lactones on the OH-proton chemical shift values in 20 and 60% (v/v) EtOH-H<sub>2</sub>O solutions. In 60% (v/v) EtOH-H<sub>2</sub>O solution, D-(-)-pantolactone and γ-octanolactone seem to promote the proton exchange between water and ethanol, since the two OH peaks coalesced to a single peak at 0.03 mol dm<sup>-3</sup> of the lactones (the OH signal of ethanol is not displayed in the figure). Moreover, the merged peak shifted further to lower field with increasing concentration of the lactones up to 0.2 mol dm<sup>-3</sup>. However, we can notice that the merged peak shifted toward higher field for >0.2 mol dm<sup>-3</sup> lactones in 60% (v/v) EtOH-H<sub>2</sub>O solution. Note that even NaCl, “a structure-breaking salt”, has given very similar effects in 60% (v/v) EtOH-H<sub>2</sub>O solution (11). In 20% (v/v) EtOH-H<sub>2</sub>O solution, D-(-)-pantolactone caused only the higher field chemical shifts. In other words, the lactone gives the structure-breaking effect in 20% (v/v) EtOH-H<sub>2</sub>O solution.

Sugar components also increased in whiskey during maturation in a cask. Glucose is one of the main sugars contained in aged whiskeys (14). The amounts of glucose detected in sherry-cask whiskeys increased remarkably in accordance with the K<sup>+</sup> concentration, suggesting that the glucose of such extremely high concentrations had come from mainly sherry components soaked in the oak wood (e.g., 44.5, 295, and ~2700 ppm of glucose at 44.7, 74.2, and 106 ppm of K<sup>+</sup>, respectively). As a whole, however, sugars gave no apparent strengthening effect of the hydrogen-bonding structure in 20% (v/v) EtOH-H<sub>2</sub>O solution (**Figure 8**).

The effects of the main flavor components, that is, esters, in whiskey on the OH-proton chemical shift were examined in 90% (v/v) EtOH-H<sub>2</sub>O solutions because of their low solubilities in water. Ethyl caproate, ethyl caprylate, isoamyl, and ethyl acetates did not cause any low-field chemical shift in the OH-proton signal of H<sub>2</sub>O or EtOH. The effects of higher alcohols, isoamyl and *n*-propyl alcohols, in 90% (v/v) EtOH-H<sub>2</sub>O were similar to those of the esters.

In the present study, we demonstrated that the hydrogen-bonding structure of water-ethanol in whiskey was strengthened by compounds that came from casks, such as acids, (poly)-phenols, and aldehydes. We may conclude that a long aging time is essential in order to gain appropriate components for “stronger hydrogen-bonding structure” as well as “the good flavor and taste” and not to stabilize physically the interaction



**Figure 8.** OH-proton chemical shifts in 20% (v/v) EtOH-H<sub>2</sub>O mixtures containing various saccharides: (○) glucose; (●) maltose; (Δ) γ-cyclodextrin; (▲) D-sorbitol.

between water and ethanol molecules. As the next step, it is definitely needed to investigate “a receptor of taste” physiologically in order to demonstrate the direct relation between the reduction of alcoholic stimulation and the structural change of water-ethanol.

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