Interactions between Ethyl Esters and Aroma Compounds in Model Spirit Solutions

John M. Conner,* Alistair Paterson, and John R. Piggott

Centre for Food Quality, Department of Bioscience and Biotechnology, University of Strathclyde, 131 Albion Street, Glasgow G1 1SD, Scotland

Activities of alcohols and long-chain aldehydes in 23% ethanol were found to be additive with esters, and limits of solubility were similar. Where the solubility limit was exceeded and agglomerates were formed, the composition of this second phase depended on the relative mole fractions and activity coefficients of the solutes present. In single solutions, addition of wood extract increased the activity coefficients of aldehydes and alcohols, but in model spirit solutions wood extract had little effect on either activity. Ester activities were influenced by aldehydes, alcohols, and wood extract, with short-chain aldehydes and wood extract decreasing overall activity. Consequently, the activity or headspace concentration of a hydrophobic aroma compound in an alcoholic beverage can be determined by the concentration and nature of other hydrophobic compounds present. Also, the dissolution of wood extract during maturation influences the distribution of the compound between the agglomerate phase and the solution.

INTRODUCTION

Freshly distilled whiskeys and brandies generally have unacceptable sensory characteristics and are traditionally matured in oak casks for several years to produce a premium product (Philp, 1989). Maturation reactions are complex, but the dissolution of wood components is of prime importance, so the character of a matured Scotch whiskey can be related to the concentrations of nonvolatile compounds (Clyne et al., 1993; Piggott et al., 1993) and it is possible to predict sensory scores for mature characteristics from quantifications of nonvolatile compounds. The loss of immature characteristics occurs as a parallel process. However, losses of ethanol and water through the cask wood increase the spirit concentrations of many volatile components during maturation (Reazin, 1983; Nishimura et al., 1983).

For the assessment of flavor, distilled spirits are typically diluted to 22 or 23% ethanol by volume to reduce pungency (Hardy and Brown, 1989; Perry, 1989). Dilution, however, changes the solubility of many volatile compounds that are more soluble in ethanol than water, such as ethyl esters of fatty acids. Further, these esters are amphiphilic, with a polar head and a hydrophobic hydrocarbon chain, and may thus form agglomerates or micelles in aqueous ethanolic solutions (Tanford, 1980; Conner et al., 1994). Salo et al. (1972) identified ethyl esters of fatty acids, notably those with even numbers of carbons between 6 and 12 in the hydrocarbon side chain, as major contributors to whiskey flavor. Jounela-Eriksson (1981) reported that addition to or depletion of these esters in spirits had negative effects on overall odor intensity.

Previous studies of the behavior of ethyl esters in redistilled brandies have shown that tannic acid and oak wood extracts significantly reduced the activity of ethyl esters in solution (Piggott et al., 1992). Conner et al. (1994) have shown, from measurements of the activity of ethyl esters, that ethyl dodecanoate and ethyl hexadecanoate are the primary components of agglomerates formed in diluted distillates. Addition of oak wood extract to esters in 23% ethanol was found to

Table 1. Composition of the Commercial Limousin Oak Extract (after Dilution to 23% Ethanol)[a]

pH	5.5
total phenols (mg of gallic acid equiv mL^{-1})	0.7
gallic acid (mg L^{-1})	0.4
vanillic acid (mg L^{-1})	0.1
vanillin (mg L^{-1})	0.2
syringic acid (mg L^{-1})	0.2
syringaldehyde (mg L^{-1})	0.5

[a] Analytical methods from Conner et al. (1992).

increase the proportion of esters in the agglomerate phase and decrease the solution concentration.

Whiskey, however, contains many other compounds in addition to ethanol and ethyl esters, whose behavior may be affected by the presence of wood extract or ester agglomerates. This paper describes a preliminary investigation of the distribution of alcohols, aldehydes, and acids between solution and agglomerate phases. A model spirit solution was prepared containing ethyl decanoate, ethyl dodecanoate, and ethyl hexadecanoate (20 mg L^{-1} each at 23% ethanol), and the distribution of compounds between the solution and ester agglomerates was studied using solute activity in the presence and absence of wood extract components.

MATERIALS AND METHODS

Materials. Ethanol was of HPLC grade (Rathburn Ltd., Walkerburn, U.K.), and water was distilled and filtered using a Millipore Q system. Ethyl decanoate, ethyl dodecanoate, ethyl hexadecanoate, dodecanol, and tetradecanol were >97% pure (Sigma Chemical Co., Poole, Dorset, U.K.). Hexanol, octanol, decanol, and valeric, hexanoic, and octanoic acid were >98% pure (BDH Ltd., Poole, Dorset, U.K.). Hexanal, octanal, decanal, and dodecanal were >97% pure (Aldrich Chemical Co. Ltd., Gillingham, Dorset, U.K.). Limousin oak extract was supplied by International Flavours and Fragrances (Haverhill, Suffolk, U.K.) and redissolved overnight in 65% v/v ethanol and filtered. The composition of the solution is given in Table 1.

Table 2. Cumulative Percentage of Compounds Detected in Three Subsequent Blank Injections after Sampling Headspace above Pure Solute (Blank 3 Taken as 100%)

| compound	analyte	blank 1	blank 2
octanol	95.6	98.4	99.4
decanol	89.3	97.7	99.3
dodecanol	69.7	88.8	96.0
tetradecanol	62.4	85.4	95.0
octanal	94.2	97.9	99.2
decanal	87.8	94.2	95.6
dodecanal	65.3	86.4	94.6

Methods. Glass vials (20 mL) were fitted with PTFE-lined silicone septa in plastic screw caps. Standard concentrations of alcohols, aldehydes, and acids were prepared in 65% v/v ethanol; 3.5 mL was placed in a vial and diluted with 6.5 mL of water to a final ethanol concentration of 23% v/v (pH 6.6). Standard concentrations were also prepared for solutions containing ethyl esters (20 mg L^{-1} each of ethyl decanoate, ethyl dodecanoate, and ethyl hexadecanoate at 23% ethanol), wood extract (2.5 g L^{-1} at 65% ethanol), and ethyl esters plus wood extract. Vials containing pure solute (10 mL) were also prepared. Solutions were equilibrated in a water bath at 30 °C for at least 30 min, and a 2.5 mL sample of headspace was withdrawn using a 5 mL gastight syringe, heated to 50 °C. Only one headspace injection was made per vial, and samples were analyzed in quadruplicate using a Carlo Erba HRGC 5300 Mega series gas chromatograph (Fisons Instruments Ltd., Crawley, U.K.) with an FID linked to a Trio computing integrator. A 0.5 mm × 12 m BP1 column (df = 1) (SGE U.K. Ltd., Milton Keynes, U.K.) was used with a helium carrier gas flow rate of 5 mL min^{-1}. A cold on-column injector was fitted with an external gastight septum. The column was at 60 °C for 1 min after injection, increasing to 240 °C at 18 °C min^{-1}. The detector temperature was 250 °C.

The glass surface in syringes can adsorb higher boiling compounds that would result in the retention of a proportion of the sample within the syringe after injection. The extent of this adsorption for C_8–C_{14} compounds was calculated from three subsequent blank injections after the headspace above the pure solute was sampled. The results, expressed as cumulative percentages, are given in Table 2. Although the proportion retained by the syringe was high for C_{12} and C_{14} compounds, it was constant, irrespective of the amount of analyte present, and so did not result in significant error in the calculation of activity. Flushing syringes with air three times prior to sampling and three times after injection greatly reduced carry-over between injections (<5% for tetradecanol), and no consistent increase was observed over four consecutive replicates.

Activities were calculated from headspace concentrations, with unit activity taken as the headspace concentration above the pure solute (Grant and Higuchi, 1990). At least five points on the linear portion of the plot of activity against concentration, expressed as mole fractions, were used to calculate the activity coefficient (Denbigh, 1981).

RESULTS AND DISCUSSION

Alcohols. Activity coefficients for alcohols are listed in Table 3. In 23% v/v ethanol the linear portions of the activity curves (Figure 1) extrapolated close to the
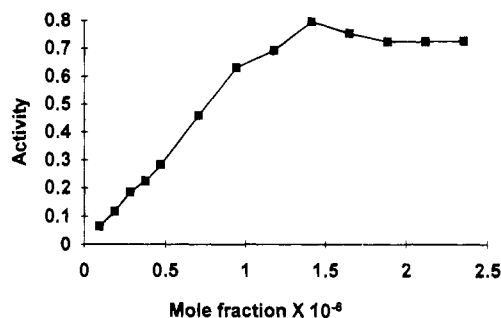
Figure 1. Activity of increasing concentrations of dodecanol in 23% v/v ethanol calculated from headspace concentration at 30 °C.

Table 3. Activity Coefficients for Homologous Series of Alcohols in Solutions at 23% v/v Ethanol at 30 °C, with Intercept and R^2 for Linear Portion of Plot

alcohol	activity coefficient[a] mean (SD)	intercept mean (SD)	R^2
Ethanol Solution			
hexanol	0.18 (0.01)	0.00 (0.00)	0.998
octanol	2.2 (0.2)	0.00 (0.00)	0.991
decanol	37 (4.9)	−0.02 (0.02)	0.966
dodecanol	650 (41)	−0.31 (0.06)	0.997
tetradecanol	5100 (670)	0.06 (0.04)	0.966
Ethanol Solution with Ethyl Esters			
hexanol	0.17 (0.01)	0.00 (0.00)	0.996
octanol	2.0 (0.1)	0.00 (0.00)	0.997
decanol	35 (3.8)	0.01 (0.02)	0.997
dodecanol	160 (23)	−0.01 (0.05)	0.979
tetradecanol	680 (72)	0.15 (0.08)	0.974
Ethanol Solution with Wood Extract			
hexanol	0.20 (0.02)	0.00 (0.00)	0.996
octanol	2.0 (0.1)	0.00 (0.00)	0.997
decanol	66 (3.8)	−0.05 (0.02)	0.997
dodecanol	370 (8)	0.02 (0.01)	0.999
tetradecanol	2800 (190)	0.22 (0.11)	0.994
Ethanol Solution with Ethyl Esters and Wood Extract			
hexanol	0.21 (0.02)	0.00 (0.00)	0.994
octanol	1.7 (0.11)	0.00 (0.00)	0.996
decanol	53 (8.9)	−0.04 (0.04)	0.973
dodecanol	200 (6.4)	−0.04 (0.01)	0.999
tetradecanol	2200 (200)	0.15 (0.07)	0.985

[a] × 10^3.

origin. This did not indicate an ideal solution, as activity coefficients were greater than 1. For dodecanol and tetradecanol, activity reached a plateau at approximately 0.7, at solution concentrations of 11 and 1.4 mg L^{-1}, respectively (Figure 1). For the homologous series of alcohols in 23% ethanol, the logarithm of the activity coefficient and the number of carbons in the alcohol gave a linear relationship with gradient = 1.31 [standard deviation (SD) = 0.4], intercept = −2.66 (SD = 0.47), and R^2 = 0.997 (Figure 2). This gave an increase in the excess chemical potential of solution (Denbigh, 1981) of 3298 J mol^{-1} CH_2. Similar results were obtained for a homologous series of ethyl esters, where the plateau region was found to be due to the formation of agglomerates (Conner et al., 1994). The point where the linear portion of the activity plot and the plateau cross represents the saturation concentration of singly dispersed molecules (Shinoda, 1978).

In the presence of ethyl ester solution but absence of wood extract, activity coefficients for dodecanol and tetradecanol were significantly lower ($p < 0.01$) than in 23% ethanol alone. For the homologous series, the increase in the excess chemical potential of solution was 2644 J mol^{-1} of CH_2, significantly lower ($p < 0.05$) than
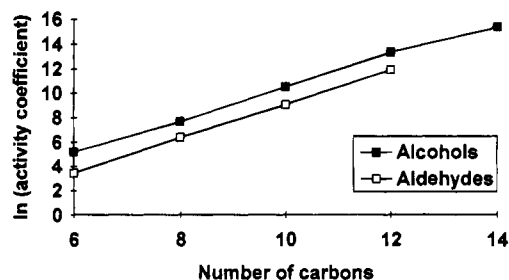
Figure 2. Logarithm of activity coefficients of homologous series of normal alcohols and aldehydes, calculated from headspace concentrations above 23% v/v ethanol at 30 °C, as a function of carbon chain length.

for 23% ethanol. This indicated that a portion of the alcohol was incorporated into the ester agglomerate phase.

For decanol, in 23% v/v ethanol in the absence of esters but presence of wood extract, the activity coefficient was significantly higher ($p < 0.05$) than in 23% ethanol alone. For dodecanol and tetradecanol, however, activity coefficients were significantly lower ($p < 0.05$). Again, a similar effect has been observed for esters (Conner et al., 1994). For C_{12} and C_{14} esters, low concentrations of wood extract increased activity coefficients and decreased the concentrations at which agglomerates were formed. Increasing either the concentration of wood extract or the ester chain length substantially decreased the concentrations at which agglomerates were formed. This suggests that the concentrations of dodecanol and tetradecanol used in activity coefficient determinations were above that required for the development of the agglomerate phase in the presence of wood extract.

No significant reductions in the activity coefficients of alcohols were observed through the addition of wood extract to ethyl ester solutions. However, in the presence of hexanol and octanol, addition of wood extract significantly decreased the sum of ester activities ($p < 0.01$). In the presence of dodecanol and tetradecanol, wood extract significantly increased the sum of ester activities ($p < 0.05$) but had no effect in the presence of decanol.

Addition of hexanol, octanol, and decanol to the ethyl ester solutions had little effect on the activities of ethyl esters independent of the wood extract. Dodecanol and tetradecanol displaced esters from solution to the agglomerate phase, decreasing the sum of ester activities at a rate equal to the increase in alcohol activity. This resulted in no overall change to the sum of solute activities.

Aldehydes. Activity coefficients for aldehydes are listed in Table 4. Again, the linear portions of the activity curves extrapolated close to the origin, but activity coefficients were greater than 1. For the homologous series of aldehydes, the logarithm of the activity coefficient and the number of carbons in the alcohol gave a linear relationship with gradient = 1.40 (SD = 0.04), intercept = −4.94 (SD = 0.20), and R^2 = 0.9997 (Figure 2). This corresponded to an increase in the excess chemical potential of solution of 3525 J mol^{-1} of CH_2.

In the ethyl ester solutions in the absence of wood extract, the activity coefficient for dodecanal was significantly lower ($p < 0.1$) than in 23% ethanol alone. The increase in excess chemical potential per mole of CH_2 in ethyl ester solution without wood extract was not significantly different from that in 23% ethanol.
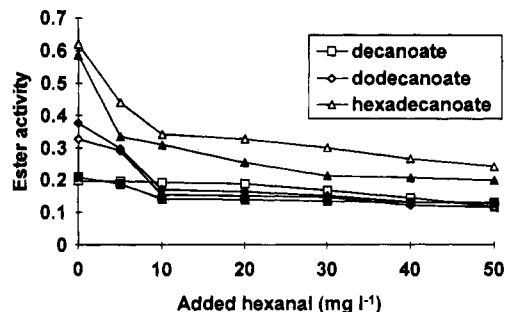
Figure 3. Ethyl ester activities, calculated from headspace concentrations above 23% v/v ethanol at 30 °C, as functions of hexanal concentration. Solid symbols represent activities in solutions containing 2.5 g L^{-1} wood extract.

Table 4. Activity Coefficients for Homologous Series of Aldehydes in Solutions at 23% v/v Ethanol at 30 °C, with Intercept and R^2 for Linear Portion of Plot

aldehyde	activity coefficient mean (SD)	intercept mean (SD)	R^2
	Ethanol Solution		
hexanal	31 (1)	0.0 (0.0)	0.999
octanal	596 (15)	0.1 (0.1)	0.999
decanal	8500 (820)	0.0 (0.0)	0.982
dodecanal	150000 (10400)	0.2 (0.3)	0.991
	Ethanol Solution with Ethyl Esters		
hexanal	31 (0.0)	0.0 (0.0)	0.999
octanal	510 (16)	0.2 (0.1)	0.998
decanal	6500 (700)	0.0 (0.1)	0.987
dodecanal	98000 (7500)	0.4 (0.6)	0.992
	Ethanol Solution with Wood Extract		
hexanal	35 (0.0)	0.0 (0.0)	0.999
octanal	770 (0.1)	0.0 (0.0)	1.000
decanal	9500 (725)	0.0 (0.0)	0.979
dodecanal	170000 (10200)	0.1 (0.3)	0.986
	Ethanol Solution with Ethyl Esters and Wood Extract		
hexanal	28 (0.3)	0.0 (0.0)	0.987
octanal	550 (60)	0.1 (0.3)	0.977
decanal	6900 (840)	0.0 (0.0)	0.959
dodecanal	12000 (9500)	0.4 (0.2)	0.989

The lower activity coefficients suggested that aldehydes were more soluble in 23% ethanol than either alcohols or esters and, except for dodecanal, were not incorporated into ester agglomerates. The lower activity coefficients for aldehydes were most probably the result of the formation of 1,1-diols, hemiacetals, and acetals in solution. In 20% ethanol at 25 °C, hemiacetal and 1,1-diol account for over 60% of the total aldehyde in solution (Perry, 1986).

For hexanal, octanal, and dodecanal, addition of wood extract to the ethyl ester solution significantly increased ($p < 0.05$) activity coefficients. Changes in the activity coefficients due to the presence of wood extract may have been caused by the decrease in pH affecting the equilibria between aldehyde, 1,1-diol, and hemiacetal.

For hexanal in ethyl ester solution, the presence of wood extract significantly lowered ($p < 0.05$) the activity coefficient. For octanal, decanal, and dodecanal, however, this was not observed. Addition of hexanal and octanal to ethyl ester solutions, both in the presence and in the absence of wood extract, decreased ester activities (Figure 3). The decrease was significantly greater ($p < 0.01$) than the increase in aldehyde activity. Addition of decanal decreased the sum of ester activities at a greater rate than the increase in aldehyde activity except in the presence of wood extract. Addition of dodecanal decreased the sum of ester activities at a

2234 *J. Agric. Food Chem.,* Vol. 42, No. 10, 1994

Conner et al.

similar rate to that of the increase in aldehyde activity, whether wood extract was present or absent.

However, in the presence of hexanal and octanal, addition of wood extract decreased the sum of ester activities, with combined reductions reaching a minimum at activity of 0.4. With decanal, no change in ester activities was observed, whereas with dodecanal, addition of wood extract yielded an increase.

This suggested that the solubility behavior of aldehydes has two components: a hydrophobic component due to the hydrocarbon chain and common to esters, alcohols, and aldehydes; and an effect due to the aldehyde group. In a spirit with an agglomerate phase formed from esters or alcohols, shorter chain aldehydes reduce the total activity or chemical potential of the solution. It is possible, therefore, that the effect of wood extract may be due to the presence of aromatic aldehydes such as vanillin and syringaldehyde.

Acids. In the concentration ranges found in whiskey, valeric, hexanoic, octanoic, and decanoic acids could not be detected in the headspace above either 23% ethanol or ethyl ester solutions buffered to pH 4.5 with 200 mM sodium acetate. No interactions with ethyl esters were observed at concentrations typical of those found in distilled spirits. At higher concentrations (100 mg L^{-1}) octanoic and decanoic acids increased the activity of ethyl decanoate. However, the sodium acetate buffer reduced the activities of all three esters.

It is apparent that long-chain aliphatic alcohols, aldehydes, and esters have limited solubility in 23% ethanol. In mixed solutions each compound makes an additive contribution to the total activity. Where a solubility limit is exceeded, excess solutes form agglomerates containing a proportion of the alcohol or aldehyde. Addition of wood extract generally increased the activity coefficients of aldehydes and alcohols, but in model spirit solutions wood extract had little effect on aldehyde or alcohol activities. Changes in ester activity relate to the hydrophobicity of the aldehyde or alcohol cosolute. Both wood extract and short-chain aldehydes were found to decrease the overall activity of ester solutions; for the latter, this effect appeared to be a property of the aldehyde group.

Conclusions. Two important implications for understanding the basis of alcoholic beverage aroma and flavor emerge from this study. First, for hydrophobic aroma compounds in 23% ethanol, activity and hence headspace concentration are not solely determined by concentration but by the presence of other hydrophobic compounds in the spirit. Second, dissolution of wood extract during maturation may alter the relative activities, and hence headspace concentrations, of certain solutes, with an effect also dependent on spirit composition.

LITERATURE CITED

Clyne, J.; Conner, J. M.; Paterson, A.; Piggott, J. R. The Effect of Cask Charring on Scotch Whisky Maturation. *Int. J. Food Sci. Technol.* **1993**, *28*, 69–81.

Conner, J. M.; Paterson, A.; Piggott, J. R. Analysis of Lignin from Oak Casks Used for the Maturation of Scotch Whisky. *J. Sci. Food Agric.* **1992**, *60*, 349–353.

Conner, J. M.; Patterson, A.; Piggott, J. R. Agglomeration of Ethyl Esters in Model Spirit Solutions and Whiskies. *J. Sci. Food Agric.* **1994**, in press.

Denbigh, K. *The Principles of Chemical Equilibrium*; Cambridge University Press: Cambridge, U.K., 1981.

Grant, D. R.; Higuchi, T. *The Solubility of Organic Compounds*; Wiley: New York, 1990.

Hardy, P. J.; Brown, J. H. Process Control. In *The Science and Technology of Whiskies*; Piggott, J. R., Sharp, R., Duncan, R. E. B., Eds.; Longman: London, 1989; pp 235–263.

Jounela-Erickson, P. Predictive Value of Sensory and Analytical Data for Distilled Beverages. In *Flavour '81*; Schreier, P., Ed.; de Gruyter: Berlin, 1981; pp 145–164.

Nishimura, K.; Ohnishi, M.; Masuda, M.; Koga, K.; Matsuyama, R. Reactions of Wood Components during Maturation. In *Flavour of Distilled Beverages: Origin and Development*; Piggott, J. R., Ed.; Ellis Horwood: Chichester, U.K., 1983; pp 241–255.

Perry, D. R. Whisky Maturation Mechanisms. In *Proceedings of the Second Aviemore Conference on Malting, Brewing and Distilling*; Campbell, I., Priest, F. G., Eds.; Institute of Brewing: London, 1986; pp 409–412.

Perry, D. R. Odour Intensities of Whisky Compounds. In *Distilled Beverage Flavour: Recent Developments*; Piggott, J. R., Paterson, A., Eds.; Ellis Horwood: Chichester, U.K., 1989; pp 200–207.

Philp, J. Cask Quality and Warehouse Conditions. In *The Science and Technology of Whiskies*; Piggott, J. R., Sharp, R., Duncan, R. E. B., Eds.; Longman: London, 1989; pp 264–294.

Piggott, J. R.; Conner, J. M.; Clyne, J.; Paterson, A. The Influence of Non-volatile Constituents on the Extraction of Ethyl Esters from Brandies. *J. Sci. Food Agric.* **1992**, *59*, 477–482.

Piggott, J. R.; Conner, J. M.; Clyne, J.; Paterson, A. Effects on Scotch Whisky Composition and Flavour of Maturation in Oak Casks with Varying Histories. *Int. J. Food Sci. Technol.* **1993**, *28*.

Reazin, G. H. Chemical Analysis of Whisky Maturation. In *Flavour of Distilled Beverages: Origin and Development*; Piggott, J. R., Ed.; Ellis Horwood: Chichester, U.K., 1983; pp 225–240.

Salo, P.; Nykänen, L.; Soumalainen, H. Odour Thresholds and Relative Intensities of Volatiles Aroma Components in an Artificial Beverage Imitating Whisky. *J. Food Sci.* **1972**, *37*, 394–398.

Shinoda, K. *Principles of Solution and Solubility*; Dekker: New York, 1978.

Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Wiley: New York, 1980.