Effect of Interface in Model Food Emulsions on the Volatility of Aroma Compounds

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Volatility of two aroma compounds—ethyl butanoate and ethyl hexanoate—was investigated in monophasic and biphasic systems. Monophasic systems were either water, an aqueous solution of sodium caseinate or sucrose stearate, or triolein. Biphasic systems, emulsified or not, consisted of triolein and one of those aqueous phases. Volatility measurements were carried out by headspace analysis and exponential dilution. In homogeneous systems, aroma retention was affected by the nature of the nonvolatile compounds: it was nearly 100% in triolein for both ethyl butanoate and ethyl hexanoate; in 5 g/L aqueous solution of sodium caseinate or sucrose stearate, retention values were 20% and 35% for ethyl butanoate and ethyl hexanoate, respectively. In biphasic systems, the vapor−liquid partition coefficient of ethyl hexanoate was not changed whether or not the system was emulsified and regardless of the nature of the surface active compound, i.e., sodium caseinate or sucrose stearate.

Keywords: *Volatility; aroma compounds; emulsions; interface*

INTRODUCTION

Acceptability of food depends on its sensory qualities and, in particular, its flavor. Aroma compounds are organic molecules (molecular mass < 400 Da) with vapor pressures sufficiently high for these molecules to be partially present in the gas state. The concentration of the free volatile substances in the gas phase depends on several factors such as their physicochemical properties, their concentration, and their interactions with the other food constituents.

Control of the aromatic quality of food requires knowledge of the nature and intensity of the interactions between aroma compounds and nonvolatile substances. That is why the formulation and flavoring of foods remain largely empirical.

Many studies have been carried out in simple systems composed of water (Buttery et al., 1969; Sadafian and Crouzet, 1986), proteins (Farès, 1987; Kinsella, 1990; Landy et al., 1995), lipids (Buttery et al., 1973; Ebeler et al., 1988), and carbohydrates (Rutschmann et al., 1989; Lebert and Richon, 1984). Nevertheless, most food products contain an emulsified lipid phase, and little work has been reported on the volatility of aroma compounds in emulsions. In physicochemical terms, particular features of emulsions are the presence and nature of the aqueous phase−lipid phase interface, the surface area of the interface, and the nature and amount of the surface active agent adsorbed at this oil−water interface. Contradictory results were reported in the literature on the effect of these various features on the partition of aroma compounds in such systems. Results were obtained by measuring the liquid−liquid or vapor−liquid equilibrium. Indeed, in emulsions, an aqueous phase and a lipid phase are dispersed, and the vapor−liquid partition of the volatile molecule depends on its liquid−liquid partition in each phase. Therefore, the measurement of the vapor−liquid partition represents an indirect means to study the behavior of the aroma substances in emulsion. Land (1978) determined the concentration of volatile molecules in vapor phase in equilibrium with an oil−aqueous phase medium, which was emulsified or not; he obtained differences in volatile concentration depending on whether the system was emulsified or not. Wedzicha (1988) put forward the hypothesis of an effect of the presence of molecules (surfactant or protein) adsorbed at the oil−water interface, say the surface of lipid droplets. This can modify the partition of aroma molecules between the aqueous and lipid phases. Le Thanh (1992) and Dubois (1994) did not notice any significant effect of the state of dispersion of an oil−aqueous phase system on the volatility of the aroma compounds. King and Solms (1979) and Dumont (1985) investigated the aroma-binding capacity of a protein added to emulsions, stabilized by either proteins or emulsifiers. These authors, respectively, did and did not obtain the presence of an effect of the nature of the surface active agent on the aroma-binding capacity of the protein.

The objective of this study was to further investigate the effect of both the nature of the surface active agent and the specific surface area in emulsions on the volatility of aroma molecules. Model food emulsions were made by homogenizing triolein with an aqueous phase containing either sodium caseinate or sucrose ester as emulsifier. Measurements of vapor−liquid partition equilibrium were perfomed for two aroma compounds: ethyl butanoate or ethyl hexanoate. These measurements were first made for a monophasic liquid system: triolein, water or an aqueous phase of sodium caseinate or sucrose stearate. Other measurements of vapor−liquid equilibrium were carried out for oil−aqueous phase biphasic systems, emulsified or not, prepared with the liquid phases previously described.

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0021-8561/96/1444-0526$12.00/0

Results are discussed in terms of the nature of the liquid phases, i.e. oil phase and aqueous phase. The effect of the specific surface area on the vapor−liquid equilibrium values was also considered.

MATERIALS AND METHODS

Preparation of Model Solutions. *Aqueous Phases.* Sodium caseinate and sucrose stearate (sucrose stearate-palmitate ester, HLB 15) were purchased from Unilait (Paris, France) and Sisterna (Sisterna SP 70; Roosendaal, the Netherlands), respectively.

Sodium caseinate and sucrose stearate were dissolved at a concentration of 5 g/L in distilled water at 60 °C by using a stirring bar. Sodium azide was added at a concentration of 0.2 g/L, and the aqueous phases were stored at 4 °C prior to initiation of experiments to inactivate microorganisms.

Lipid Phase. Triolein [1,2,3-tri(*cis*-9-octadecenoyl)glycerol; 65% purity] was purchased from Sigma Chemical Co. (St. Louis, MO) and purified by percolating it through a column packed with magnesium silicate ($MgO:SiO_2 \approx 15:85$) of particle size 0.150−0.250 mm (60−100 mesh ASTM) (Florisil; Merck, Darmstadt, Germany) (Gaonkar, 1989). The purified triolein was stored at 4 °C prior to initiation of experiments.

Lipid-Containing Systems. Nonemulsified systems were prepared by pouring triolein on the aqueous phase contained in a tube to obtain two separated layers. Oil-in-water emulsions were prepared by homogenization of the liquid lipid phase with an aqueous phase containing sodium caseinate or sucrose stearate using a jet homogenizer under an inlet pressure of 4×10^5 Pa (Model LPJH-134; Labplant, Huddersfield, U.K.). The volumetric fraction determined by picnometry was 15% (v/v). The emulsions were stored at 4 °C prior to initiation of experiments.

Solubility Index Measurement. The solubility index of sodium caseinate was determined in triplicate according to the method of Bastier et al. (1993).

Emulsion Characterization. A Malvern Mastersizer laser diffractometer (Model S2-01; Malvern Instruments, Worcs, U.K.) was used to determine the droplet size distribution, from which was derived the volume surface average diameter d_{32} and the surface area (Courthaudon et al., 1992). Droplet size distribution was also determined after measurements of vapor−liquid equilibrium.

Observations of emulsions were made with a phase contrast microscope (Leitz Labovert, Germany) to show the possible presence of aggregates of oil droplets (magnitude ×400).

Protein Content Determination. The systems, emulsified or not, were centrifuged at 15000*g* for 20 min. The protein concentrations of the aqueous phases were measured in triplicate by using the Lowry method (Lowry et al., 1951) with bovine serum albumin as standard (Prolabo, Marly-le-Roy, France).

Sodium caseinate concentrations in the aqueous phase (obtained after centrifugation) of nonemulsified systems and in emulsions were 5.4 ± 0.3 and 4.5 ± 0.2 g/L, respectively. The absorbance measurement of 5 g/L sodium caseinate solution permitted the determination of the correction factor with reference to bovine serum albumin. This factor is necessary because of a different composition in amino acid residues of the two proteins. By calculating the difference in protein concentration between the original aqueous phase and the emulsion aqueous phase (supernatant), we obtained the amount adsorbed at the surface of triolein droplets. In a nonemulsified system, the interfacial surface area was too low to determine the amount of protein adsorbed at the interface.

Flavoring. Ethyl butanoate and ethyl hexanoate were purchased from Prolabo (Marly-le-Roy, France) and Aldrich (Strasbourg, France), respectively. The systems were equilibrated at 25 °C before the aroma compounds were added. For concentrations ranging from 20 to 5000 ppm (v/v) of aroma compounds (according to the solubility of the volatile molecules in the medium), the corresponding volume of the volatile compound was carefully added to a tube containing 20 mL of medium, and the tube was tightly capped. Before analysis, the homogeneous and lipid-containing systems were equilibrated at 25 °C; for the latter, at least 1 h of regular agitation was necessary to reach an equilibrated repartition of the aroma compound between the different phases.

Measurement of the Vapor−Liquid Equilibrium. The method used to measure the vapor−liquid equilibrium was the headspace analysis or exponential dilution coupled with gas−liquid chromatography. An inert gas (nitrogen) passed through the liquid phase at a constant flow rate (from 1.5×10^{-5} to 10.0×10^{-5} m^3/min according to the medium) and carried the volatile compound into the headspace. A sample of the vapor phase (1 mL) was automatically injected into the gas chromatograph at regular intervals.

The chromatograph was equipped with a flame ionization detector (Chrompack CP 9000; Chrompack Co., Middelburg, The Netherlands) and with a 3 m stainless steel column (inner diameter 2.2 mm) packed with Chromosorb W-AW 100−200 mesh Carbowax 20 M-10%. The operating parameters of the chromatograph were as follows: injector temperature, 190 °C; detector temperature, 200 °C; column temperature, 100 °C; N_2 flow rate, 1.6×10^{-5} m^3/min; H_2 flow rate, 2.5×10^{-5} m^3/min; air flow rate, 25×10^{-5} m^3/min. The chromatograms were registered and treated with the Chroma software (Biosystèmes, Couternon, France).

The obtained data permitted the determination of the vapor−liquid partition coefficient of aroma compounds at infinite dilution. This value represents their volatility. By headspace analysis, the vapor−liquid equilibrium is considered to be reached when the peak area of the aroma compound in the gas phase is constant. The vapor−liquid partition coefficient expressed in concentration (h_i^∞) is the ratio of the volumetric concentration c (ppm) of the aroma compound i in vapor phase to the volumetric concentration in liquid phase (eq 1):

$$h_i^\infty = c_{\text{vapor}}/c_{\text{liquid}} \tag{1}$$

Exponential dilution consists of exhausting the liquid phase of aroma compounds in equilibrium with the vapor phase. The solute chromatographic peak area variation is an exponential function of time provided the detector response is linear (eq 2) (Sorrentino et al., 1986):

$$\ln S = \ln S_0 - (pd/RTN)K_i^\infty t \tag{2}$$

S and S_0 are the volatile peak areas; d is the carrier gas flow rate (m^3/min); R is the gas constant ($R = 8.314$ J/K per mol); T is the temperature (K); p is the total pressure (Pa); N is the number of moles of liquid phase; K_i^∞ is the vapor−liquid partition coefficient of the aroma compound (in molar fraction); and t is the time (min).

K_i^∞ is calculated from the values of the slope (a) of the straight line obtained by plotting ln S against time (eq 3):

$$K_i^\infty = -aRTN/pd \tag{3}$$

K_i^∞ and h_i^∞ are related by the following equation:

$$h_i^\infty = K_i^\infty \frac{M_{\text{liq}} p}{RTd_{\text{liq}}} 7.5 \times 10^3 \tag{4}$$

M_{liq} is the molar mass of the liquid phase and d_{liq} the density of the liquid phase.

The partition coefficient of the aroma compounds (eq 1) at 760 mmHg and 25 °C is denoted h_{760}^∞ and determined in water, triolein, 5 g/L sodium caseinate (or sucrose stearate) solution, and biphasic systems emulsified or not. The volatility of the aroma compounds in water was chosen as a reference and permitted the determination of the percentage of retention r (eq 5). Therefore, a significant variation of h_{760}^∞ ($P = 0.05$) in comparison with the reference was due to an aroma−medium interaction.

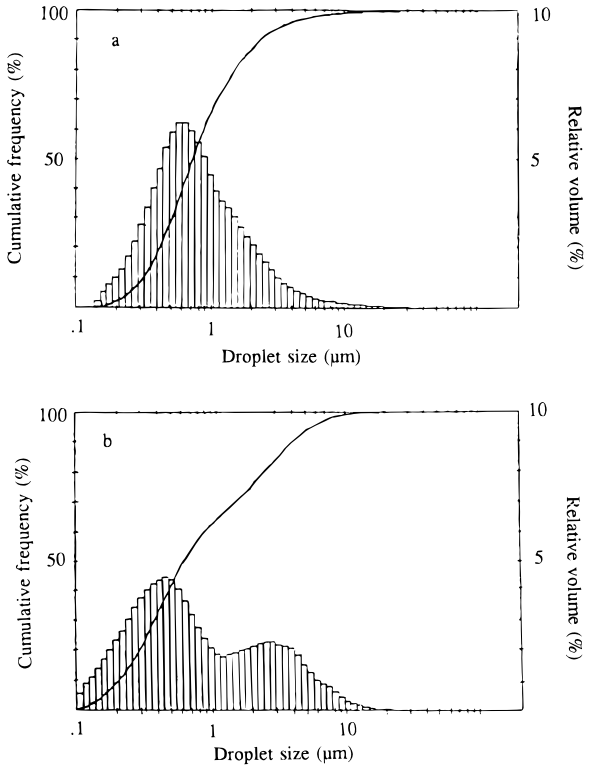

Figure 1. Droplet size distribution of two types of 85/15 oil-in-water emulsions (performed at room temperature): (a) 5 g/L aqueous solution of sodium caseinate; (b) 5 g/L aqueous solution of sucrose stearate.

$$r = [1 - (h^{\infty}_{760\ \text{system}}/h^{\infty}_{760\ \text{water}})] \times 100 \qquad (5)$$

$h^{\infty}_{760\ \text{system}}$ is the volatility of the aroma compound in a homogeneous system.

Effect of the Nature and Surface Area of Oil−Water Interface. To understand how the surface area and nature of the aqueous phase−oil interface influence the volatility of ethyl butanoate and ethyl hexanoate, a two-level factorial experiment (abbreviated 2^2 design) was used. The two factors were X_1, degree of triolein dispersion, and X_2, nature of the interface; the two levels were "no" and "yes" for X_1 and "sodium caseinate" and "sucrose stearate" for X_2. A complete factorial design was performed, so that four experiments were done in triplicate.

RESULTS

Physicochemical Characterization of the Medium. *Sodium Caseinate Solution.* The solubility index of sodium caseinate in solution at 5 g/L was 0.90 g/g of dry solids at 25 °C. This means that sodium caseinate is mostly dissolved in the protein solutions prepared.

Lipid-Containing Systems. For nonemulsified systems, the specific surface area was 1.6×10^{-3} m^2/mL of triolein. Figure 1 shows the droplet size distribution in the oil-in-water emulsions. Distribution of fat droplets was unimodal when the surface active agent was sodium caseinate and bimodal when sucrose stearate was used. In the emulsions, the mean specific surface area was 10.5 m^2/mL of triolein, regardless of the composition of the aqueous phase (aqueous solution of sodium caseinate or sucrose stearate at 5 g/L).

From droplet size measurements, no change in droplet size or distribution was noticed before or after vapor−liquid equilibrium determinations. Moreover, observations by phase contrast microscopy did not show any formation of oil droplet aggregates.

Protein Surface Coverage at the Triolein Aqueous Phase Interface. From protein content determinations in the aqueous phases of emulsions, the protein surface coverage was 0.5 mg/m^2 of interface.

Volatility of Aroma Compounds. The vapor−liquid partition coefficients and the percentages of retention of ethyl butanoate and ethyl hexanoate in triolein or aqueous phases are given in Table 1. The volatility of the two aroma compounds was much lower in triolein than in aqueous mediums; values were lower in the presence of a surface active compound than in water alone. The volatility of ethyl butanoate was not significantly different ($P \geq 0.05$) between the sodium caseinate and sucrose stearate solutions. On the contrary, that of ethyl hexanoate was significantly different ($P \leq 0.05$) between those two aqueous phases: the volatility of ethyl hexanoate is lower in a 5 g/L solution of sucrose stearate than in a 5 g/l solution of sodium caseinate. In aqueous phases, the volatility of ethyl hexanoate was higher than that of ethyl butanoate; however, the reverse was observed in the presence of triolein. Consequently, when the hydrophobicity of the aroma compound increased, the volatility observed in aqueous phases increased and that in lipid phases decreased.

Table 2 gives the volatility values of each aroma compound in emulsified or nonemulsified systems as a function of the nature of the aqueous phase. The volatility of ethyl hexanoate was affected neither by the nature of the surface active agent present nor by the state of dispersion of the system. However, for ethyl butanoate, volatility values were lower in the presence of sodium caseinate than in the presence of sucrose stearate, but they were not significantly different whether the system was emulsified or not. In other words, there was an effect of the nature of the surface active agent but no effect of the state of dispersion on the volatility of ethyl butanoate. Additionally, the vapor−liquid partition coefficient of ethyl butanoate (1.17×10^{-3}) was lower in the emulsions stabilized by sodium caseinate than in the nonemulsified triolein−water system (1.33×10^{-3}).

DISCUSSION

The volatility of ethyl esters in lipid-containing systems in the presence of sodium caseinate or sucrose stearate was not modified when the surface area of the liquid−liquid interface increased from 1.6×10^{-3} to 10.0 m^2/mL of triolein. Consequently, we can infer that the ethyl esters are not adsorbed at the liquid−liquid interface, or they may be but in a too weak proportion to be detected. They should mainly be solubilized in the aqueous continuous phase. An adsorption could be observed only if the aroma compounds had an affinity for the protein adsorbed at the interface, in which case the presence of a protein film with a large surface area would lower the volatility of the aroma compounds in the emulsion. We can suggest that the strong affinity of the volatile substances for triolein is too high to be able to detect any difference in volatility that could come from the nature of surface active agent present or from the surface area of the oil−water interface. Concerning biphasic systems containing sodium caseinate, the absence of an effect of the interfacial surface area can be explained by the low quantity of protein adsorbed at the liquid−liquid interface (0.5 mg/m^2 of triolein). Indeed, in the literature, determinations of the surface coverage of β-casein or sodium caseinate at triglyceride−

Table 1. Vapor−Liquid Partition Coefficients and Percentages of Retention *r* of Aroma Compounds in Aqueous Phases and Triolein[a]

	water	5 g/L sodium caseinate		5 g/L sucrose stearate		triolein	
aroma compound	h^{∞}_{760} ($\times 10^3$)	h^{∞}_{760} ($\times 10^3$)	r (%)	h^{∞}_{760} ($\times 10^3$)	r (%)	h^{∞}_{760} ($\times 10^3$)	r (%)
ethyl butanoate	13.5 ± 0.8	$12.5^{NS} \pm 0.1$	7.4	$12.2^{NS} \pm 1.0$	9.6	$0.22^{*} \pm 0.01$	98.40
ethyl hexanoate	34.0 ± 2.4	$22.0^{*} \pm 1.3$	35.3	$18.5^{*} \pm 0.7$	45.6	$0.03^{*} \pm 0.00$	99.90

[a] NS, not significant ($P \geq 0.05$); * $P \leq 0.05$.

Table 2. Effect of the Nature and Surface Area of the Liquid−Liquid Interface on the Volatility of Aroma Compounds

	parameters		h^{∞}_{760} ($\times 10^3$)[a]	
expt	X_1 = dispersion degree	X_2 = interface nature	ethyl butanoate	ethyl hexanoate
1	no	water	$1.33^{abc} \pm 0.05$	$0.16^{d} \pm 0.01$
2	no	sodium caseinate	$1.25^{a} \pm 0.05$	$0.14^{d} \pm 0.01$
3	yes	sodium caseinate	$1.17^{ac} \pm 0.06$	$0.16^{d} \pm 0.02$
4	no	sucrose stearate	$1.45^{b} \pm 0.04$	$0.15^{d} \pm 0.00$
5	yes	sucrose stearate	$1.47^{b} \pm 0.15$	$0.14^{d} \pm 0.01$

[a] Values with the same superscript are not significantly different ($P \geq 0.05$).

water interfaces ranged from 1 to 2 mg/m^2 (Courthaudon et al., 1991; Dickinson and Tanai, 1992; Tornberg, 1980). In our study, the measurement of the solubility index reflects that a part of the sodium caseinate was not dissolved and could not thus be adsorbed at the liquid−liquid interface. In the homogeneous systems, however, we verified that the aroma retention is identical in the presence or not of this nonsoluble part. To verify the possibility for the protein film to be a barrier for the transport of volatile molecules, its thickness should be changed and tested. Dubois (1994) obtained an effect of interfacial surface area of a model cheese containing calcium caseinate (from 11% to 22%) on the volatilities of diacetyl or allyl sulfide; both of their headspace concentrations decreased when the surface area of oil droplets increased. In model emulsions prepared with 1 g of calcium caseinate/100 g of solution, the surface area had no effect on volatility. Le Thanh (1992) came to the same conclusion (lack of effect of interfacial surface area) with compounds of various hydrophobicities diluted in biphasic systems containing arabic gum as emulsifier. However, Land (1978) observed a modification of the volatility of allyl isothiocyanate with the interfacial surface area: it was less volatile in an emulsion (surfactant not specified); dimethyl sulfide should reverse results. No explanation of these results could have ever been given by the authors. In their opinion, the nature of the aroma compounds and food components seems to play an important role in volatility.

Another way to study the effect of the nature of the interface is to use different surface active agents. Then their influence on the volatility of aroma compounds can be observed. The vapor−liquid partition coefficient of ethyl butanoate varied with caseinate or sucrose stearate, which were adsorbed at the liquid−liquid interface; it varied whatever the degree of dispersion. If these results were extended to food systems, the nature of the surface active agent would be a more important factor than the interfacial surface area. Nevertheless, this effect was not observed for ethyl hexanoate because its volatility was not affected by the nature of the emulsifier used. These results obtained with emulsions underscore the importance of the characteristics of aroma compounds and cannot be explained in comparison with the results obtained with aqueous media. Indeed, in aqueous media containing sodium caseinate or sucrose stearate, the volatilities of ethyl butanoate were not significantly different ($P \geq 0.05$), whereas the volatilities of ethyl hexanoate were (Table 1). Later, we intend to use different surface active agents to compare their effects on the behavior of aroma compounds and to confirm the possibility of an effect of the nature of the interface.

A physicochemical method would be necessary to estimate the interactions between an aroma molecule and lipids, proteins, or surfactants. Electron spin resonance (ESR) is suggested to label a surface active agent, i.e. a protein (Le Meste et al., 1991), and to investigate the mobility of the probe before and after aroma compounds are added to an emulsified system.

Salvador et al. (1994) found changes in the volatility and rate of release of diacetyl between oil-in-water and water-in-oil emulsions. This difference was unlikely to be due to the emulsifier because the same emulsifier was used for both emulsions to avoid the variation of a barrier at the droplets' interface. Moreover, both emulsion types had the same droplet size distribution. The results of Salvador et al. (1994) emphasize that, besides the interface−nature and surface area−the nature of the dispersed phase (aqueous or lipid phase) plays an important role in the volatility and rate of release of aroma compounds.

The measurement of the concentration of aroma compounds in the aqueous phase is another approach to study their behavior in oil-in-water emulsions. In this case, the aim is rather to investigate the flavor release into saliva than the volatile release, as a function of time and volumetric fraction of oil (Linssen et al., 1993; McNulty and Karel, 1973). This kind of work is more relevant to our understanding of the mechanisms of the flavor perception during eating (Overbosch et al., 1991). The significance of the obtained data could be checked by conducting sensory measurements of flavor release from the emulsions, as Dubois (1994) did previously.

CONCLUSION

A study of the interactions between aroma compounds and food emulsion components was carried out by headspace analysis or exponential dilution. Both methods were suited for homogeneous and biphasic systems.

In homogeneous systems, ethyl butanoate and ethyl hexanoate retentions in triolein were comparable; however, they increased in the presence of sodium caseinate or sucrose stearate, along with their hydrophobicity (low retention for butanoate and higher retention for hexanoate).

In lipid-containing systems, it appeared that the surface area and nature of the aqueous phase−oil interface did not influence the volatility of ethyl hexanoate. However, the volatility of ethyl butanoate changed with the nature of the surface active agent. This conclusion cannot be explained by using the results

obtained from the homogeneous systems. In fact, the presence of triolein, which induced interactions with proteins or with commercial emulsifiers, seems to be at the origin of complex phenomena. We also noted an effect due to sodium caseinate in an emulsion because the partition coefficient of ethyl butanoate was lower in emulsion than in a water−triolein system. Thus, the retention properties of the protein could act at the aqueous phase level, the liquid−liquid interface, or both.

Eventually, to better understand the role of aqueous phase−oil interface on the behavior of aroma compounds, it would be necessary to further study the surface active agent−oil interactions or aroma−interfacial protein interactions. The effects of such interactions on retention and transfer (through the different liquid phases) of aroma compounds could be approached via accurate physicochemical methods.

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Received for review May 9, 1995. Accepted November 3, 1995.[⊗]

JF950279G

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1996.