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Solvent Effects in Competition between Guest Molecules for β-Cyclodextrin

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Abstract. The effect was investigated of increasing concentrations of co-solvents ethanol, isopropanol, tetrahydrofuran (THF), dimethylformamide (DMF), formamide and ethylene glycol on the selectivity of inclusion for binary mixtures of the following guest molecules: pinene, eugenol, cineole, limonene and camphor, and of isopropanol for the ternary mixtures eugenol/cineole/pinene; and limonene/cineole/pinene. In contrast to the strong solvent dependent effects on β-cyclodextrin solubility, the changes in selectivity are similar for each system, show little variation from solvent to solvent and are guest dependent. Similar guest induced effects are observed for ternary mixtures. They appear to be additive.

Key words: Cyclodextrins, inclusion, terpene mixtures, co-solvent.

1. Introduction

β-Cyclodextrin is the most widely used of the cyclodextrins in the cosmetic, food and pharmaceutical industries [1]. Complex guest mixtures will be involved in many cases, for example in the encapsulation of flavours or perfumes. As both taste and odour reponses are highly sensitive to relatively small changes in relative proportions of components in the mixtures, it will be extremely useful to have available a knowledge of competitive inclusion between the compounds found in such mixtures and how this competitive inclusion varies under different conditions.

We are systematically investigating the effects of co-solutes [2] and co-solvents [3] on the solubility of the cyclodextrins in aqueous media, and we have also reported the inclusion selectivity of a range of terpenoid compounds in β-cyclodextrin [4]. We are now combining these studies to systematically report on the effects of co-solvent on inclusion selectivity in β-cyclodextrin. In a preliminary report we cited the effects of isopropanol [5]: the study is here extended to all three classes of solubility modulating solvents [3]. Class A: ethanol, isopropanol, and tetrahydrofuran. Class B: dimethylformamide. Class C: formamide. The work has been carried out for a series of binary and ternary mixtures of terpenoids. It is shown that the selectivity changes are related to the percentage of water in the mixtures,

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thus showing little or no dependence on the nature of the co-solvent, and that the variations observed are strongly dependent on the terpenes in the individual mixtures.

2. Experimental

We have adopted a standardised procedure in order to obtain a valid internal comparison of the results.

2.1. MATERIALS

Commercially available solvent (Merck, analytical grade) was used without further purification. β-Cyclodextrin was a gift from Wacker SA. The terpenoid molecules and DMSO-d_6 were purchased from Aldrich and were used without further purification. NMR spectra were recorded on a Brüker AC 200 Spectrometer (200 MHz).

2.2. METHODS

2.2.1. *Inclusion Selectivity Measurements*

β-Cyclodextrin (2.5g: 2.2×10^{-3} mol) was added to 50 mL of water–solvent mixture with a composition varying from 100% (v/v) water to 50% water–50% solvent. Equimolar terpenoid mixtures (2.2×10^{-3} mol of each) were added to the solutions under stirring and were maintained at 40°C for 1 h. The precipitated complexes thus obtained were filtered, washed with water (2×25 mL) and diethyl ether (2×25 mL) and dried under reduced pressure. The relative selectivity of the complexation of terpenes by β-cyclodextrin was measured by integration of the ^{1}H NMR spectra of the precipitated compounds in DMSO-d_6. Measured yields were in the range 75–90% with no discernible relation between guest or co-solvent proportion and yields.

The same method was used for the ternary mixture, with equimolar mixtures of three terpenoids (2.2×10^{-3} mole of each).

3. Results and Discussion

The structural formulae of the terpenoid guest molecules are given in Figure 1. We have previously demonstrated the formation of 1 : 1 host–guest complexes for each of these molecules with β-CD [4]. The following terpene derivative types were chosen: one ether, one alcohol or phenol, one monocyclic hydrocarbon, one bicyclic hydrocarbon. Partial results were obtained for one ketone derivative. These correspond to the different types of terpenoid studied in our previous work on competition in water [4].

Table I gives the solvent classes and the solvent concentration at which the maximum in the solubility of β-CD is observed. The solvents used in this paper

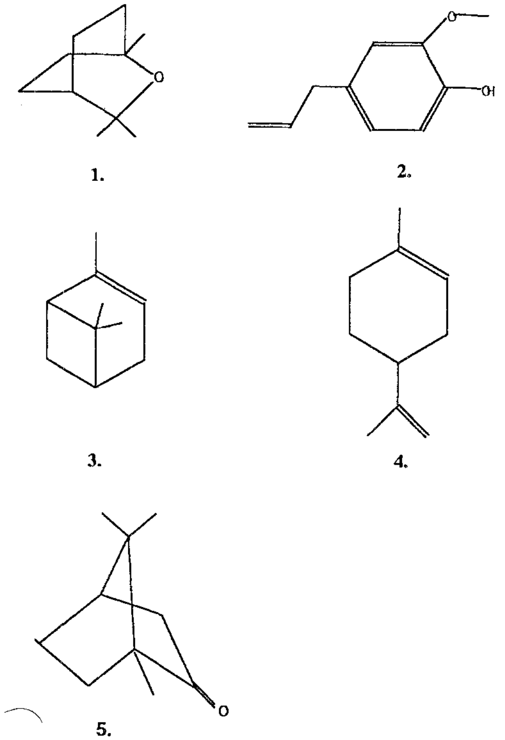

Fig. 1. Structures of the terpenoids used: **1.** Cineole, **2.** Eugenol, **3.** Pinene, **4.** Limonene and **5.** Camphor.

are in italics. Figure 2 presents schematic drawings of the typical solubility curves for the three solvent classes. The solvents were chosen to represent the various classes defined for solubility measurements. Since most solvents were in class A we chose two widely used alcohols, ethanol and iso-propanol, as well as THF for its capacity to solubilise a wide range of organic compounds. Acetone was not chosen as considerable evaporation was anticipated.

Class A solvents, which form clathrate hydrates or clathrate hydrate-like aggregates in solution [6], and include tetrahydrofuran, ethanol, and isopropanol, show an increase in solubility rising to a maximum at quite low solvent mole fractions. This maximum solubilisation corresponds to the percentage of maximum variation in thermodynamic parameters such as excess partial molar volume of the solvent [6].

Class B solvents, which form strong hydrogen bonded molecular complexes with water, include dimethylformamide. Here the solubility curves show two regions of unchanging solubility with a relatively sharp change between the values. This point corresponds to the stoichiometry of the molecular complex between water and the cosolvent, e.g. 2 : 1 H_2O: DMF [6].

TABLE I. Solubility of β-cyclodextrin in various co-solvent/aqueous mixtures. Taken from [3].

Solvent	Mole fraction for maximum	Solubility gL^{-1}
Class A		
Methanol	0 (22)[a]	18.5[c]
Ethanol	10	22
Iso-propanol	8	70
Acetonitrile	8	37
Tetrahydrofuran	5	22
Ethylene-glycol-dimethylether	2	38
Acetone	8	47
Dioxane	5	23
Class B		
Dimethylformamide	40	365
Dimethylsulfoxide	40	770
Pyridine	65	70
Ethylene-glycol	890[b]	29
Class C		
Formamide	100	400

[a] Methanol shows an inflection in the solubility at 22% corresponding to A type.

[b] Ethylene glycol shows an initially decreasing solubility followed by a plateau type behaviour.

[c] For methanol the solubility decreases in all proportions from 100% H_2O.

Class C solvents and ionic co-solutes simply show an increase in β-CD solubility with increasing mole fraction. This class is represented here by formamide.

A final solvent, ethylene glycol, seems to have a behaviour combining that of class A and class C systems.

Figures 3a, b and c show the effects of class A co-solvents on the relative inclusion selectivity of the pinene/eugenol, pinene/cineole and cineole/eugenol guest systems, the solvents being ethanol, propanol-2-ol and tetrahydrofuran, respectively. The general forms of the selectivity plots are very similar, and in consequence guest effects will be discussed first. We have previously shown, in agreement with the work of Ueno [7], that the guest selectivities in competition experiments have little or no relation to that which might be expected in terms of comparison of the association constants between individual guests and β-cyclodextrin. So our analysis here is based simply on the observed effects and we do not try to relate our results to association constants.

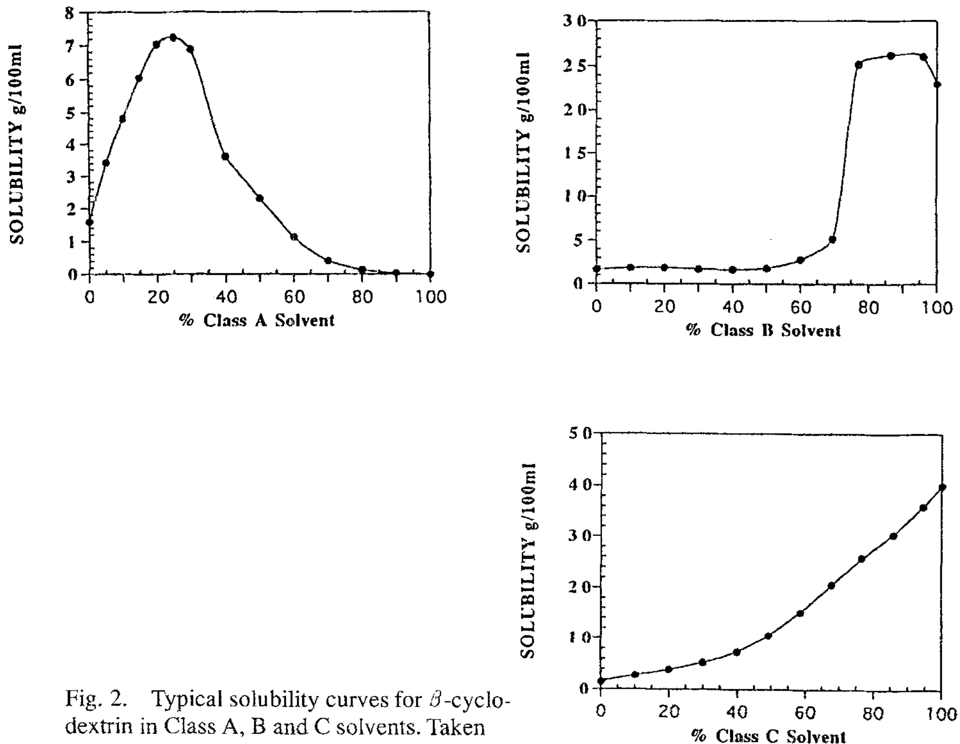

Fig. 2. Typical solubility curves for β-cyclodextrin in Class A, B and C solvents. Taken from Ref. 3.

For the two systems containing eugenol, this molecule is determinant in the product ratio found in the isolated inclusion complex as the co-solvent concentration increases. This reaches zero at 30% for propan-2-ol and THF and at 40% for ethanol, for the pinene/eugenol system. For the cineole/eugenol system the percentage for zero inclusion of eugenol is 10% for THF, 20% for propan-2-ol and 40% for ethanol. In the case of the cineole/pinene system, there is a slight initial increase in the amount of cineole present and then a slow decrease in the cineole content with increasing co-solvent, although interestingly, for propan-2-ol the percentage of cineole included increases once again above 50%. Extending the terpenoid systems for propan-2-ol as a co-solvent (Figure 3d), gives the results for the limonene/eugenol, limonene/cineole and limonene/camphor complexation experiments, respectively. Once again, with the eugenol systems at 30% co-solvent no eugenol is present in the inclusion complex. The curve for the cineole/limonene system corresponds closely to that of the cineole/pinene system. For the limonene/camphor system there is a general tendency for the percentage of camphor to decrease from 90% to a limiting value of 60% above 40% propan-2-ol.

Summarising, it would seem that the inclusion selectivity variations in the class A co-solvents are dominated by the nature of the terpenoids present in the mixture,

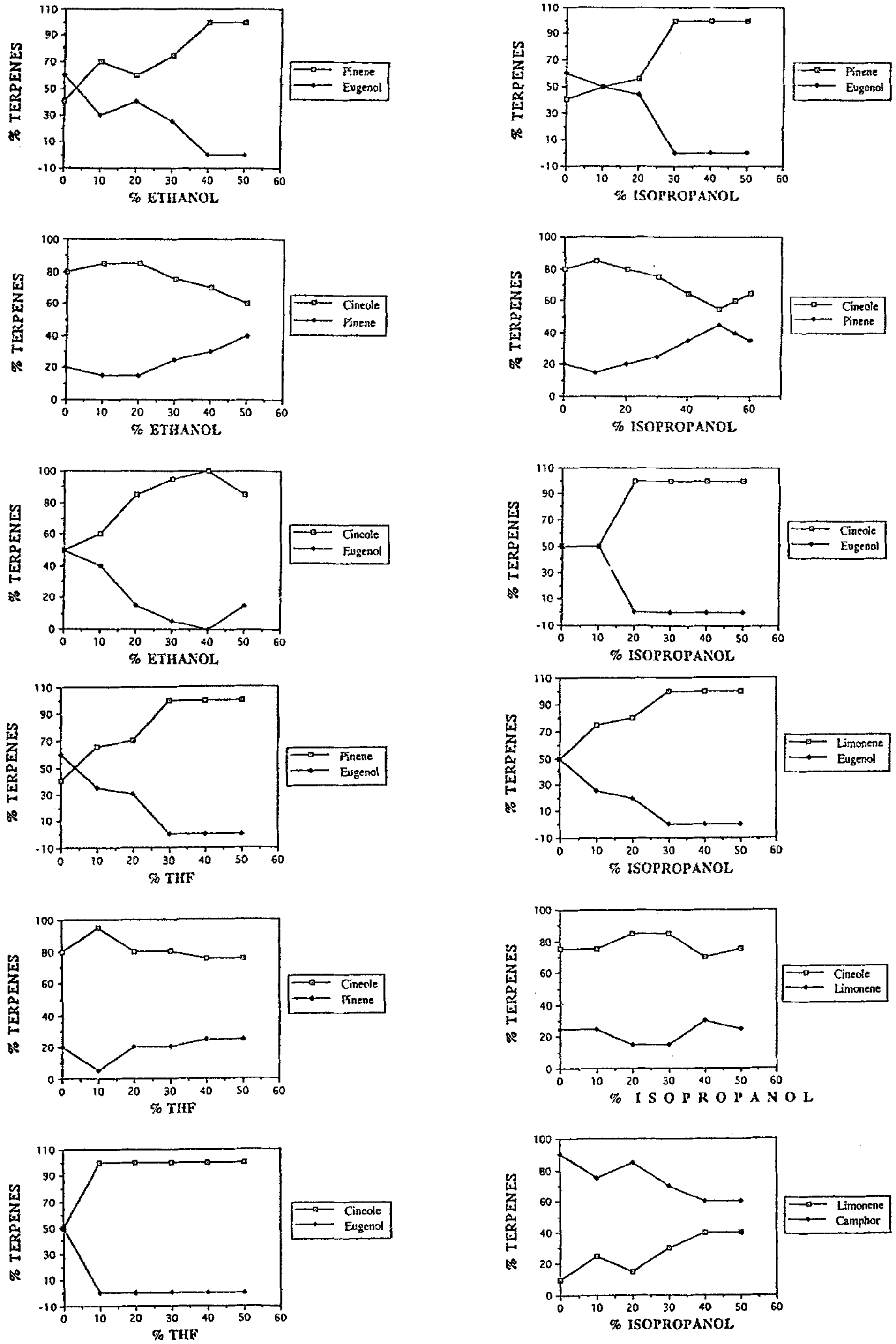

Fig. 3. Relative proportions of guests included in β-cyclodextrin in: **3a** ethanol, **3b** isopropanol, **3c** tetrahydrofuran for cineole/pinene/eugenol binary mixtures and **3d** isopropanol for cineole/limonene/eugenol and the limenone/camphor binary mixtures.

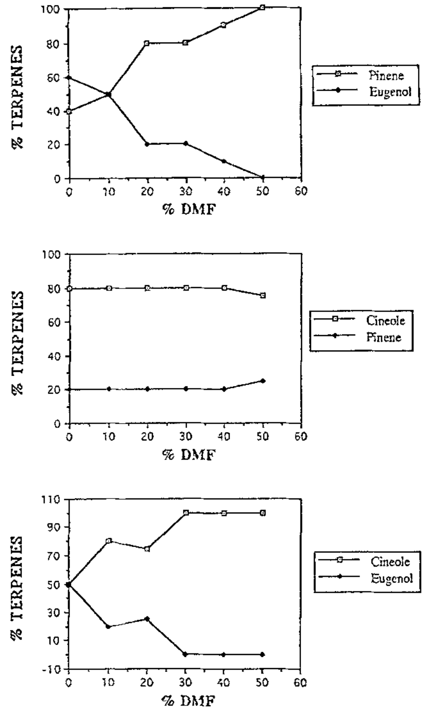

Fig. 4. Relative proportions of guests included in β-cyclodextrin in dimethylformamide for cineole/pinene/eugenol binary mixtures.

with eugenol or cineole leading to curves in which the second component has only slight effects.

The solvent-based variations seem to arise in the low co-solvent concentration area. The variations are similar but the concentration of co-solvent at which a given variation occurs shifts with a change in solvent. For example, the percentage of co-solvent at which no incorporation of eugenol occurs in the isolated product varies in the order tetrahydrofuran < propan-2-ol < ethanol. This order is in agreement with the percentage of co-solvent at which the maxima in the solubilisation of β-CD [5] occur and at which maxima are observed in the variation of such thermodynamic parameters as partial excess molar volume [6]. There is then some evidence of a small solvent specific effect.

Figure 4 shows the effects of one co-solvent of class B (dimethylformamide) on the relative inclusion selectivity of the pinene/eugenol, pinene/cineole and cineole/eugenol guest systems. For the two systems containing eugenol there is a diminution in the amount of this complex found in the inclusion product with increasing co-solvent. This reaches zero at 50% DMF for the pinene/eugenol system and at 30% DMF for the cineole/eugenol system. For the pinene/cineole system there is no variation in the inclusion product with increasing co-solvent. The effect of DMF is quite similar to the class A co-solvent effects, even though DMF, which

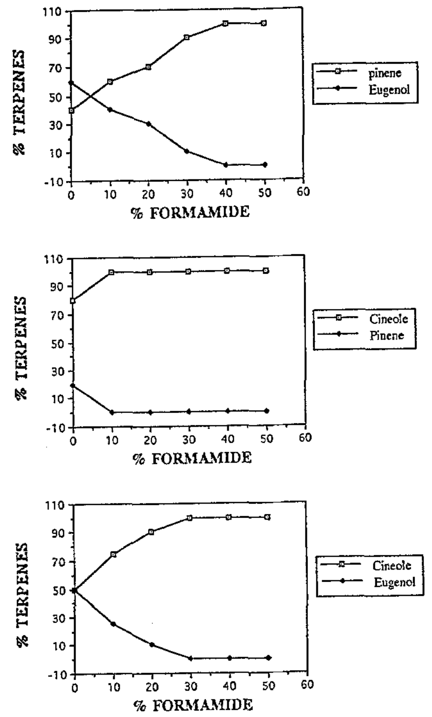

Fig. 5. Relative proportions of guests included in β-cyclodextrin in formamide for cineole/pinene/eugenol binary mixtures.

forms strong molecular complexes with water [6], gives rise to a solubility curve for β-cyclodextrin which has no similarity to the solubility curves observed for class A solvents. It would seem that the inclusion selectivity variation in the class B solvent systems is also dominated by the nature of the terpenoids present in the mixture.

Figure 5 shows the effects of one co-solvent of class C (formamide) on the relative inclusion selectivity of the pinene/eugenol, pinene/cineole and cineole/eugenol guest systems. For the cineole/pinene system there is a disappearance of pinene in the isolated complex at 10% formamide, and for the two systems containing eugenol the processes are exactly the same as those observed for class A and B co-solvents: i.e. above 30% no eugenol is present in the isolated complex. For the three systems the curves are similar to those of the class A solvents although here the variations are much smoother.

The three classes of solvent display very similar behaviour in the relative selectivity of the inclusion process, with some slight variations arising from the contributions of individual solvents. The dominant factor is probably the change in the solvent polarity relative to the guest molecules.

Figure 6 shows the selectivity curves for ethylene glycol/water mixtures. As noted before, the solubility curve for this solvent resembles those of the class

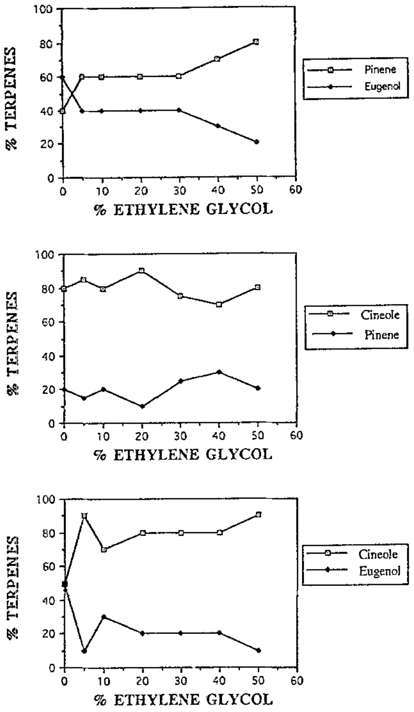

Fig. 6. Relative proportions of guests included in β-cyclodextrin in ethylene glycol for cineole/pinene/eugenol binary mixtures.

A solvents at low co-solvent concentrations (<20%) and that of class C at high concentrations. Similar behaviour is seen here, with sharp variations, as seen for the class A at low co-solvent concentrations and smoother changes above 30% as seen for formamide.

A comparison of the solubility curves for three binary mixtures eugenol/pinene, cineole/pinene and eugenol/cineole in d_8-propan-2-ol/D_2O mixtures [5], shows that, interestingly, while the first two systems mirror the inclusion selectivity, the eugenol/cineole relative solubilities show little change with increasing d_8-propan-2-ol concentration. This would rule out the effects being simply due to a preponderance of one component in the mixture. It would in fact seem that, as the solubility of a component in the solvent mixture increases, the percentage incorporated in the isolated complex decreases. This may be considered reasonable if the driving force for inclusion is solvophobicity of the guest species.

It may be proposed that significant co-solvent participation in the host–guest complex may occur, as has been observed by Warner for pyrene complexation in the presence of alcohols [8]. In order to verify this we looked for solvent peaks in the NMR spectra of unwashed but well dried products. For THF there is approximately 0.5 equivalent of solvent per guest molecule; for the other class A solvents no significant amounts of co-solvent are observed. For dimethylformamide

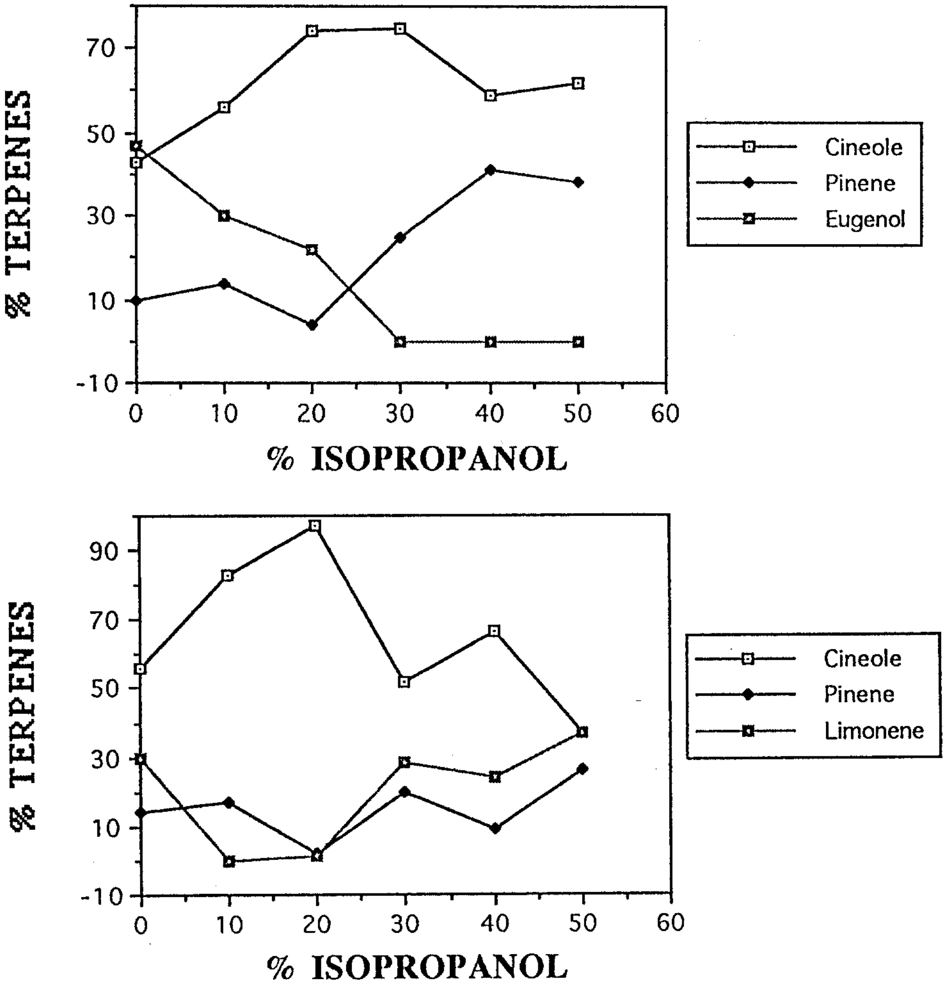

Fig. 7. Relative proportions of guests included in β-cyclodextrin in isopropanol for (a) cineole/pinene/eugenol and (b) cineole/pinene/limonene ternary mixtures.

only 0.1 equivalent of solvent per guest is observed. Such a small quantity may be explained by the low volatility of the solvent, which was not eliminated on drying. For formamide no solvent is found in the complex. It appears that little participation by the co-solvent in the host-guest complex occurs in general.

Figure 7 gives the guest composition curves for triple competition experiments: eugenol/cineole/pinene and pinene/cineole/limonene. Both series of experiments were undertaken in water/propan-2-ol mixtures. For the first mixture, eugenol again disappears from the inclusion complex above 30% propan-2-ol, and the cineole and pinene curves also resemble those of the binary mixture. For the second mixture the plot shows a good fit with those observed for the binary mixtures pinene/cineole and limonene/cineole. These data would appear to show that competition effects are simply additive and that extrapolation from binary to ternary mixtures is possible.

4. Conclusion

We have shown that changes in co-solvent concentrations can have strong effects on the relative percentages of guest included within β-cyclodextrin in the course of competitive inclusion experiments. These changes for solvents such as propan-2-ol or ethanol are quite dramatic in regions where there is relatively little co-solvent. They have important implications for the use of the co-solvents in the preparation of inclusion compounds of essential oils, where changes in the co-solvent may lead to dramatic changes in the balance of compounds giving rise to a particular taste or aroma. The apparent possibility of extending from binary to ternary mixtures suggests that application of simple curves to multicomponent systems may well be possible.

Acknowledgements

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References

1. J. Szetjli: *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht (1988).
2. A. W. Coleman and I. Nicolis: *Supramol. Chem.* **2**, 93 (1993).
3. A. W. Coleman, M. Munoz, A. K. Chatjigakis, and P. Cardot: *J. Phys. Org. Chem.* **6**, 651, (1993).
4. C. Donzé and A. W. Coleman: *J. Incl. Phenom.* **16**, 1 (1994).
5. C. Donzé, A. K. Chatjigakis, and A. W. Coleman: *J. Incl. Phenom.* **13**, 155 (1992).
6. F. Franks: *Water, A Comprehensive Treatise*, Vol. 2, Plenum Press, London (1973), and references therein.
7. A. Ueno, T. Kuwabara, A. Nakamura, and F. Toda: *Nature* **356**, 136 (1992).
8. A. Muños de la Peña, T. Ndou, J. B. Zung, K. L. Greene, D. H. Live, and I. M. Warner, *J. Am. Chem. Soc.* **113**, 1572 (1991).