Supercritical Fluid Extraction, a Novel Method for Production of Enantiomers

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Extraction of 25 different binary mixtures of racemic acids [2-(4-isobutylphenyl)propionic acid, *cis*- and *trans*-chrysanthemic, and -permetric acid] and various chiral bases with supercritical carbon dioxide permitted the conclusion that molecular chiral differentiation in a supercritical fluid is more efficient than in conventional solvents. In the majority of cases, however, complete separation could not be achieved. In five cases, remarkable partial resolutions were realized (30−75% ee) and resolution was possible on a preparative scale. The pair *cis*-chrysanthemic acid and (*S*)-(+)-2-(benzylamino)-1-butanol was studied in detail. Pressure, temperature, time, as well as the molar ratio of base and acid had a marked influence on the quantity and quality of the products. Increasing pressure or decreasing temperature resulted in higher ee values. (−)-*cis*-Chrysanthemic acid in 99% ee was obtained from the raffinate in a single extraction step. Multiple extractions produced the (+)-*cis*-acid in 90% ee.

Introduction

Separation of enantiomers is invariably based on differences induced by diastereomeric interactions. While resolution through direct crystallization of diastereomeric salts, complexes, or compounds is still the most important approach,[1] other methods, such as sublimation[2] or distillation,[3] have been occasionally successfully applied as an alternative. Chiral chromatography is currently the most popular and most effective resolution method, mainly on an analytical scale. Supercritical fluid chromatography (SFC), a recently developed method using a chiral complexing agent bonded to a stationary phase, enables reliable and accurate analytical separation for several important types of compounds[4−7] (α-amino acids, α-hydroxy ketones, barbiturates, diols, β-blockers), as well as of many other biologically active molecules.[8−17] SFC on packed columns is also suitable for separation of the enantiomers on a preparative scale.[18−20] Owing to short analysis times and efficient separation SFC on packed columns successfully competes with conventional HPLC,[21,22] but concerning the amount of material that can be separated it has the same limitations.

Efficiency of SFC on packed columns suggested that supercritical extraction (SFE) may also be developed to an optical resolution method applicable on a preparative and perhaps even on an industrial scale. To our knowledge, chiral separation using SFE has not been reported.

In a preliminary publication,[23] we reported that for some model compounds molecular chiral differentiation supercritical carbon dioxide is much more efficient for the separation of enantiomeric mixtures than traditional solvents. In SFE with carbon dioxide extraction parameters (pressure, temperature, and extraction time) can be conveniently optimized. SFE has the additional advantage that it is carried out at low temperature and does not cause racemization or thermal decomposition of the compounds. Upscaling may not cause fundamental problems, since nowadays large-scale SFE units are in use for the decaffeination of green coffee beans or tea, as well as for the extraction of hop, spices, and tobacco.[24,25]

Owing to their different scalar physical properties, the separation of diastereomers is, of course, possible by SFE. Thus, SFE can be a powerful alternative to conventional resolution techniques mentioned above.[1−3] The purpose of the present study was to demonstrate the usefulness of this method and to investigate the influence of some parameters upon the resolution process.

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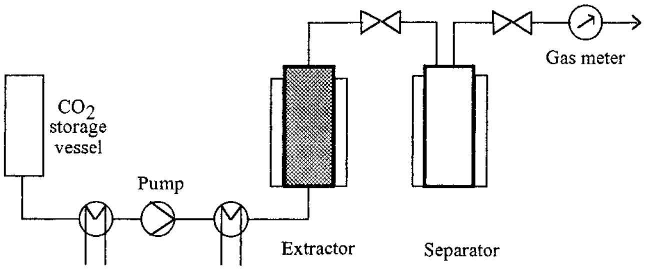

Figure 1. Flow diagram of the laboratory equipment.

Table 1. Racemic Compounds and Resolution Agents

no.	racemic compds
1	(±)-2-(4-isobutylphenyl)propionic acid
2a	(±)-*cis*-chrysanthemic acid
2b	(±)-*trans*-chrysanthemic acid
3a	(±)-*cis*-permetric acid
3b	(±)-*trans*-permetric acid

no.	resolution agents
4	(*R*)-(+)-1-phenylethylamine
5	(*R*,*R*)-(−)-1-(*p*-nitrophenyl)-2-aminopropane-1,3-diol
6	(*S*)-(+)-2-amino-1-butanol
7	(*S*)-(+)-1-phenyl-*N*-methylisopropylamine
8	(*S*)-(+)-2-(benzylamino)-1-butanol

Experimental Section

Materials and Sample Preparation. Both the racemic mixtures of various acids and the resolving agents (see Table 1) were prepared in our laboratory and their purity (>98%) checked by analytical methods (NMR, melting point). Other analytical grade reagents were obtained from Reanal Ltd., Budapest, and Carlo Erba Reagenti. The samples were prepared by mixing various chiral bases with racemic acids in a 0.5:1 molar ratio. All possible 25 acid−base combinations were studied. Perfilt, a porous supporting material, was impregnated with the mixtures, placed into the extractor vessel, and extracted with supercritical carbon dioxide of 99.5% (w/w) purity supplied by Messer Griesheim Hungaria.

Optical rotations of extracts and raffinates was measured on a Perkin-Elmer 241 polarimeter. Enantiomeric excess (ee) was determined from optical rotation on the basis of calibration with enantiomeric mixtures of known purity.

Apparatus and Extraction. Figure 1 shows the schematic flow diagram of the laboratory-scale equipment. First, the extractor is filled with the sample. Liquid carbon dioxide is supplied from a cylinder and released into the inner storage vessel. It is compressed to the desired pressure by means of the pump and heated to a specified extraction temperature in order to bring it into the supercritical state before it is passed through the extraction vessel filled with the sample. Once the system has attained the required temperature and pressure, carbon dioxide is allowed to flow through the vertically mounted bed. The effluent is then expanded through a micrometering valve into the separator, where the extract precipitates. The volume of the evaporated carbon dioxide is measured with a dry gas meter. Finally, the system is depressurized, the residue removed from the extractor, and the raffinate is recovered by simple solvent extraction. The apparatus enables us to weigh either the total extract or individual fractions withdrawn at certain time intervals. Extraction was done at 40 °C and at least 90 bar. The pressure was increased up to 250 bar if the amount of extract was not satisfactory at lower pressure.

Results and Discussion

Molecular Chiral Recognition in Supercritical Carbon Dioxide. In our experiments, we used the well-established technique[26] of resolving the racemic mixture (in our case acids) with less than 1 equiv of the resolving agent (in our case an optically active base). In this way, a complex equilibrium is established involving both enantiomers of the free acid and two diastereomeric salts. Our resolution procedure was based on two distinct phenomena, the second one being specific to SFE: (i) The higher stability of one of the diastereomeric salts results in its preferred formation. Consequently, the free acid becomes optically active. (ii) The poor solubility of the salts in supercritical carbon dioxide permits the selective extraction of the free acid. These two phenomena are interdependent since the dissociation constant of the diastereomeric salts, an important factor influencing the optical purity of the extract, is also dependent on the state of the solvent (pressure and temperature).

A series of SFE carried out with different racemic acids and resolving agents has shown that in fact salts of the bases **5**−**7** with all of the acids studied were practically insoluble in carbon dioxide, while those of **4** and **8** were only slightly soluble. With the latter, the extracts were contaminated with some salt carried over with the solvent. Table 2 records the configuration and ee values of the major extracted enantiomer. In the case of fractionated extraction, the average enantiomeric excess of subsequent extracts is given in Table 2. With combinations **1**−**4**, **2a**−**8**, **3a**−**4**, **3a**−**5**, and **3b**−**8** and for seven more pairs **1**−**5**, **2a**−**5**, **2a**−**6**, **2b**−**8**, **3a**−**7**, **3b**−**4**, and **3b**−**5** resolution was significant, while those pairs where resolution was under the detection limit were omitted from the table. The overall weight yields (sample preparation, extraction, and product recovery) were in the range of 60−90%.

Conventional resolution of the same racemic acids with the listed bases has been described in the literature; namely: four acid + base pairs in water (**1** + **4**,[28] **2a** + **8**,[29] **2b** + **8**,[29] **3a** + **4**,[30]), two pairs in water−acetone mixtures (**3a** + **8**,[29] **3b** + **8**[29]), one pair in a 2-propanol−petroleum ether mixture (**1** + **5**[27]), and in a water−2-propanol mixture (**1** + **4**[28]).

From our experiments, we concluded that for the separation of enantiomeric mixtures supercritical carbon dioxide is much more selective than conventional solvents. For the pair **2a** + **8** we have studied the process in detail.

Detailed Study of the Resolution of Racemic *cis*-Chrysanthemic Acid (2a) with (*S*)-(+)-2-(Benzylamino)-1-butanol (8) by SFE. Solubility in Supercritical Carbon Dioxide. First, the solubility of the individual components was determined at the same parameters used in resolution experiments. Dissolved material was proportional to the solvent passed through the vessel. The solubilities of **2a** and **8** in carbon dioxide at 39 °C and 100 bar are 0.36 wt % and 0.17 wt %, respectively, while Perfilt proved to be insoluble.

Resolution by SFE. Mixtures of (*S*)-(+)-2-(benzylamino)-1-butanol (**8**) and racemic *cis*-chrysanthemic acid (**2a**) in a 0.5:1 molar ratio were prepared and extracted with supercritical carbon dioxide at 37 °C and 100 bar.

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Table 2. Configuration and Enantiomeric Excess of Extracts and Raffinates (for the Free Acid)

		base									
		4		5		6		7		8	
acid		confgn	ee (%)	confgn	ee (%)	confgn	ee (%)	confgn	ee (%)	confgn	ee (%)
1	extract:	*S*	33	*R*	8						
	raffinate:	*R*	42	*S*	11						
2a	extract:			*R*	2	*R*	4			*R*	43
	raffinate:			*S*	2	*S*	4			*S*	76
2b	extract:									*S*	3
	raffinate:									*R*	3
3a	extract:	*R*	40	*R*	15			*S*	2		
	raffinate:	*S*	48	*S*	38			*R*	2		
3b	extract:	*S*	9	*S*	13					*S*	27
	raffinate:	*R*	3	*R*	10					*R*	31

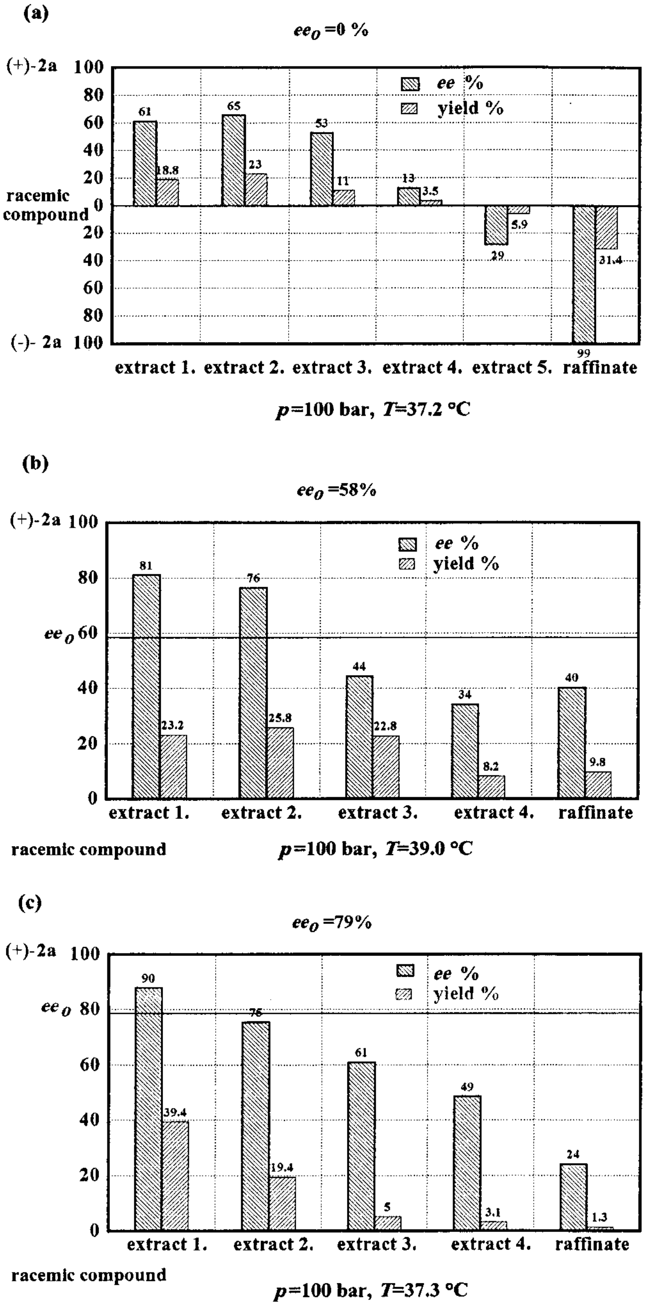

Figure 2. Resolution of racemic *cis*-chrysanthemic acid (**2a**) by multiple SFE using (*S*)-(+)-2-(benzylamino)-1-butanol (**8**): (a) enantiomeric excess and overall yield of the subsequent extract samples and the raffinate obtained by single extraction (the corresponding carbon dioxide consumption is given in Figure 4), (b) second extraction, (c) third extraction.

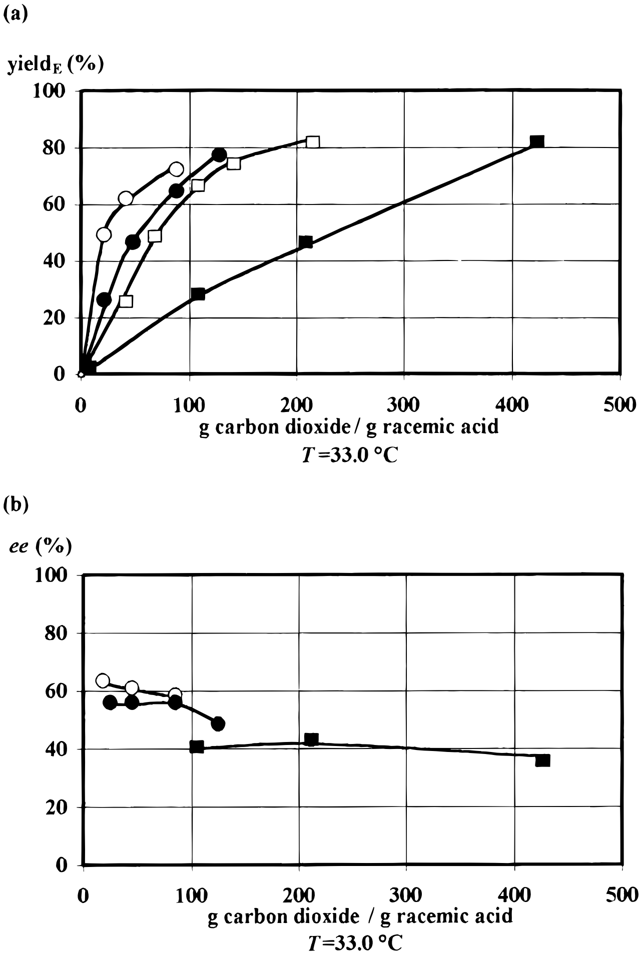

Figure 3. Effect of the pressure (a) on the yield of extract and (b) on the enantiomeric excess (pressure (bar): ○ 150, ● 120, □, 100; ■, 90).

At certain time intervals and/or passing a certain volume of carbon dioxide the eluted amount of material was weighed. The extracts contained the (+)-enantiomer of the free acid and some salt, whereas the raffinate was the mixture of diastereomeric salts. After a single extraction, (−)-enantiomer recovered from the raffinate was essentially optically pure (ee 99%) (Figure 2a). Ee values and yields of the first three fractions were more or less constant, but dropped significantly in fraction 4, while in fraction 5 the (−)-enantiomer predominated. Combination of fractions 1−3 gave the (+)-enantiomer in 58% ee and 53% yield. Further purification of the partially resolved acid (**2a**) was possible by repeated extractions after the addition of base **8** equimolar with the content of (−)-**2a** until a satisfactory resolution was achieved (90% ee). Figure 2 summarizes the results obtained by multiple extractions. Experiments similar

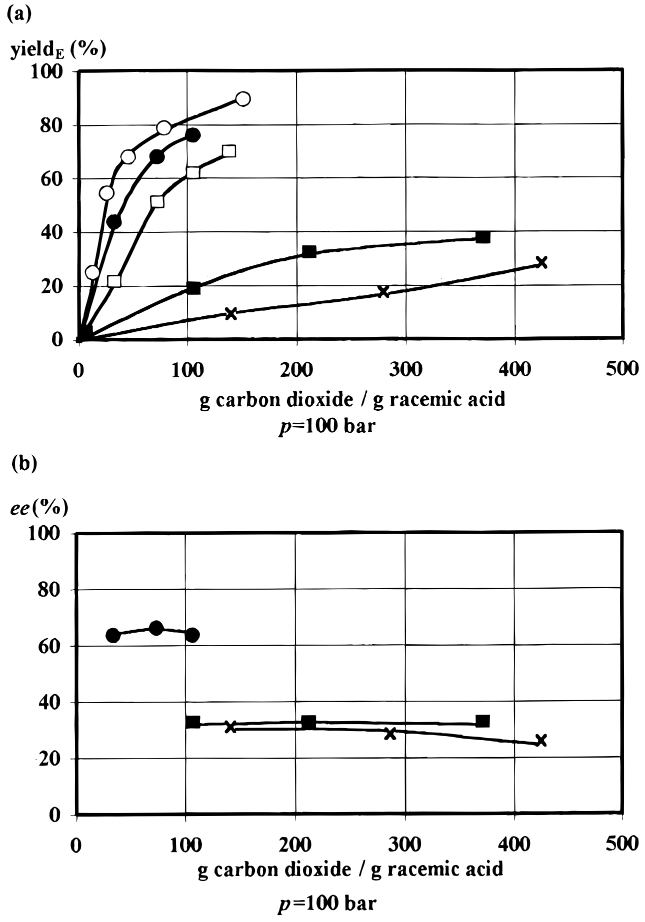

Figure 4. Effect of the temperature (a) on the extraction curve and (b) on the enantiomeric excess (temperature (°C): ○ 32, ● 35, □ 37, ■ 47 × 50).

to that shown in Figure 2a permitted us to determine the optimal time of extraction.

Effects of Pressure and Temperature. Dissociation of the diastereomeric salts and the dissolving power of carbon dioxide can be influenced by extraction temperature and pressure. The pressure of carbon dioxide (in the range of 90−150 bar) was found to have a direct bearing on yield and enantiomeric excess (Figure 3). Yield increased at higher pressure. The enantiomeric excess of the extracts obtained from a single extraction at constant temperature (33 °C) increased from 41% to 62% by increasing the pressure from 90 to 150 bar.

The effect of temperature was investigated only in the supercritical state (in the range of 32−50 °C) of carbon dioxide. All the extractions were carried out at 100 bar (Figure 4).

The ee values of the extracts obtained after a single extraction were 25% and 65% at 50 and 32 °C, respectively. Thus, selectivity of extraction with carbon dioxide decreased with increasing temperature.

Resolution in supercritical carbon dioxide is a complex function of pressure and temperature. For a better understanding of the process, ee values of the total extracts obtained from a single extraction were plotted against the density of the fluid (Figure 5).

Below 0.6 g/cm^3, the enantiomeric excess is nearly linear with carbon dioxide density and approaches a constant value in the higher density region. However, in the range of 0.6−0.85 g/cm^3, the results are scattering and ee values are significantly higher at lower temperatures (32−35 °C). Further studies are needed to enable predictions at higher densities.

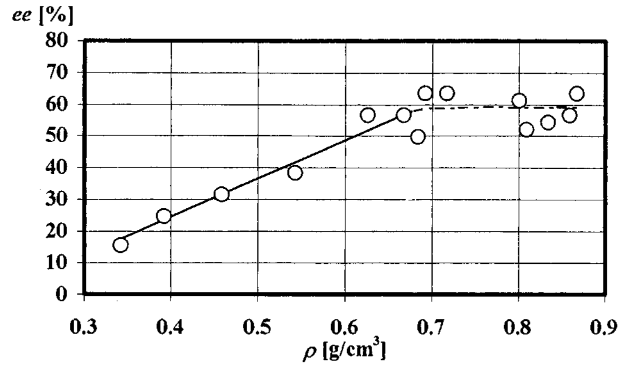

Figure 5. Effect of the carbon dioxide density on the enantiomeric excess of the total extracts obtained by single extraction.

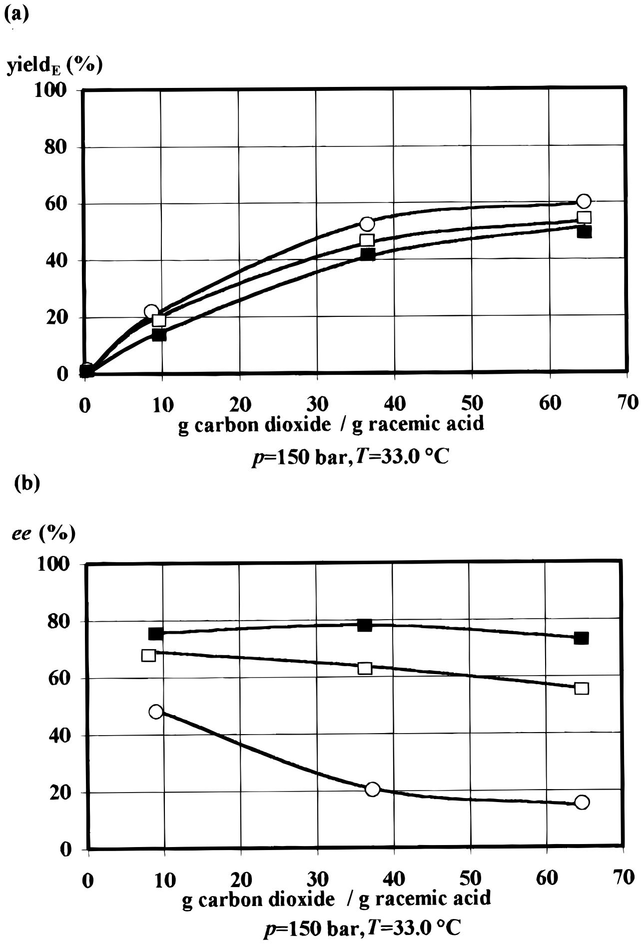

Figure 6. Effect of the molar ratio of **8** and **2a** (a) on the yield of extract and (b) on enantiomeric excess (molar ratio: ○ 0.25, □ 0.50, ■ 0.75).

Effect of Molar Ratio. In our standard experiments, the racemic acid was allowed to react with 0.5 equiv of the chiral bases. Extractions at 0.25, 0.50, and 0.75 molar ratios of **8**/**2a** have shown that an increase of the molar ratio resulted in enhanced enantiomeric excess but reduced yields (Figure 6).

Improving the Enantiomeric Purity of Partially Resolved Mixtures. Sodium hydroxide in an equimolar amount was added to the racemic fraction to the partially resolved acid obtained by a single extraction. In Figure

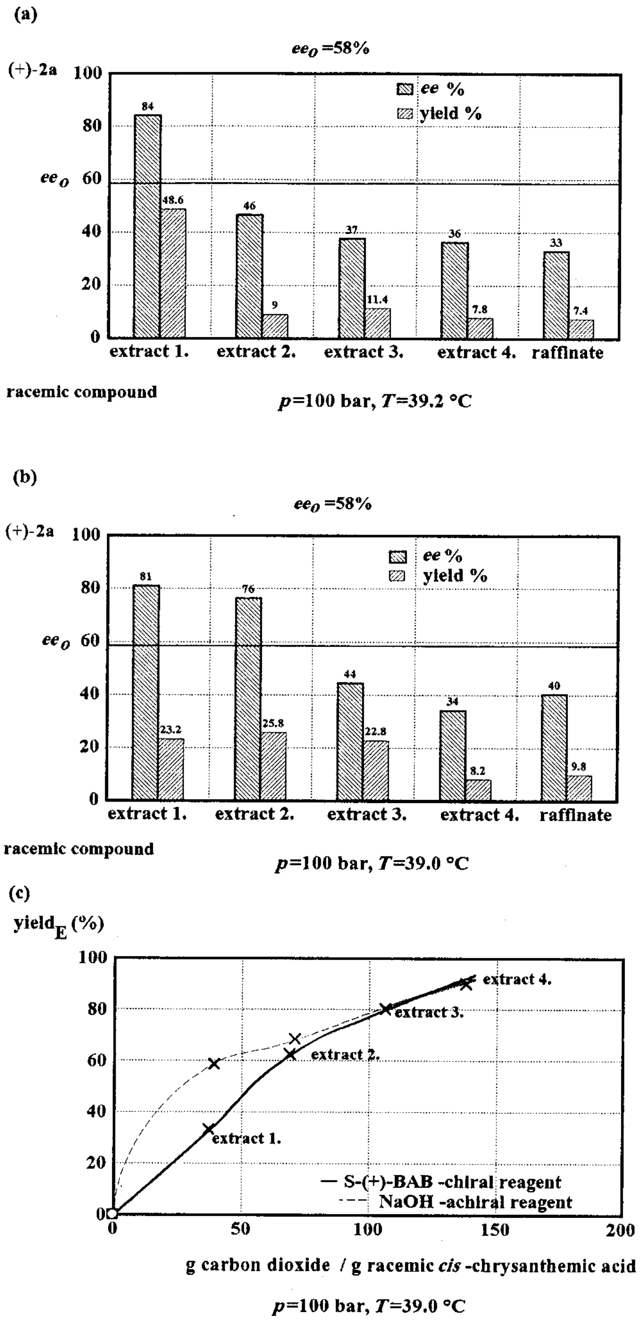

Figure 7. Comparison of resolutions enantiomeric mixture of *cis*-chrysanthemic acid (**2a**) by (a) using achiral (sodium hydroxide) and (b) chiral reagent [(*S*)-(+)-2-(benzylamino)-1-butanol (**8**)]; (c) comparison of the extraction curves.

7, the use of a chiral and an achiral agent for upgrading partially resolved **2a** is compared. The achiral version gave somewhat higher yields and ee values.

Conclusions

SFE is a new and promising technique for the resolution of some racemic acids. Some reasons for choosing carbon dioxide as a supercritical fluid over other solvent systems are as follows: (i) the unique solvation and favorable mass transfer properties of supercritical fluids in general, which can be varied simply by adjustment of pressure and/or temperature; (ii) carbon dioxide is nontoxic, nonexplosive, readily available, and easily removed from the products to be recycled; (iii) the critical temperature of carbon dioxide is 31 °C, which allows operation close to ambient temperatures, avoiding thereby decomposition or racemization of thermally labile substances; and (iiii) carbon dioxide is cheaper than organic solvents.

Due to high operating pressures, supercritical fluid technology is potentially hazardous, and safety must be taken into account in equipment design, installation, and operation. However, the combination of strict inspection procedures, high design standards, and official tests enforced by state agencies limits this hazard to a minimum.[31]

Our results demonstrate that differences in solubility, dissociation constants, and stability are larger in supercritical carbon dioxide as compared to traditional solvents. Exploitation of these differences may lead to novel and more efficient methods for optical resolution. SFE also offers cleaner products, less sample handling, and equivalent or better recoveries than conventional technologies.

As it was demonstrated by the resolution of the (±)-*cis*-chrysanthemic acid, pressure, temperature, time and resolving agent can influence the extraction process. Investigation of the density dependence of solvating power revealed that below 0.6 g/cm^3 the enantiomeric excess of the extracts increased linearly with carbon dioxide density, while further studies are needed to be able to predict the behavior of the system at higher densities. In contrast to preparative packed column SFC and preparative HPLC, by SFE several grams of material can be conveniently resolved for bioassays in less time. The process is applicable to both laboratory and large-scale use.

Preparative Experiments. Resolution of (±)-*cis*-Chrysanthemic Acid (2a). Racemic **2a** (1.68 g, 10 mmol), (*S*)-(+)-2-(benzylamino)-1-butanol (**8**) (0.9 g, 5 mmol), Perfilt (2.0 g), and methanol (10 mL) were mixed and then evaporated. The residue was extracted with supercritical carbon dioxide. The temperature of the extract was set at 40 °C, and the quantity of carbon dioxide used for extraction was 150−200 g depending on the enantiomeric mixture. After the removal of the carbon dioxide, (+)-**2a** (0.8 g, $[\alpha]^{20}_{D} = +48.5°$, $c = 10$ mg/mL, chloroform) was obtained. To the raffinate were added methylene chloride (20 mL) and 5 N hydrochloric acid (10 mL), and the aqueous phase was further extracted with methylene chloride (2 × 20 mL). The combined organic phases were evaporated to give (−)-**2a** (0.8 g, $[\alpha]^{20}_{D} = -63.4°$, $c = 10$ mg/mL, chloroform).

Results obtained by a similar procedure carried out with 10 mmol of racemic **1, 2b**, **3a**, and **3b** and 5 mmol of **4**−**8** are given in Table 2.

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