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Enantiomeric Separation of Chiral Components Reported To Be in Coffee, Tea, or Cocoa

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Literally hundreds of volatile compounds have been identified in coffee, tea, and cocoa. A significant proportion of these compounds are known to be chiral. The enantiomeric composition of most of these compounds is unknown, and until recently there were no efficient analytical procedures for their identification. The advent of cyclodextrin-based chiral stationary phases for capillary gas chromatography provides effective means for the enantioselectivity analysis of many of these compounds. Some of these are known to be flavor and fragrance components that occur naturally or are produced from the manufacturing process (e.g., fermentation, drying, and roasting).

INTRODUCTION

Recently, the importance of enantiomeric separations to food and beverage analysis has attracted increasing attention (Armstrong et al., 1990a). In fruits, many of the chiral constituents responsible for flavoring and aroma are natural products and are present in the unprocessed pulps and juices. Recently, Guichard et al. (1990) reported that C_6–C_{12} γ-lactones were responsible for the unique aroma of apricots. The 4*R* enantiomers of the γ-lactones were found to predominate in apricots and strawberries, but significant amounts of some of the 4*S* enantiomers were found as well (Guichard et al., 1990; Mosandl et al., 1990a). Hence, full characterization of aroma profiles may require quantitation of the enantiomeric purities of the chiral constituents. In addition, the enantiomeric composition of fruit juices and other consumable products may be useful in the detection of adulteration (Kuneman et al., 1988).

Many of the constituents of raw tea, coffee, and cocoa are amino acids and sugars which are not particularly volatile (Blanc et al., 1989; Rohan and Stewart, 1967; Offem, 1990; Sakata et al., 1987; Ohtsuki et al., 1987). However, it is known that many volatile constituents are formed during processing (Baltes and Bochmann, 1987; Rohan and Stewart, 1966). Some of these compounds result from the degradation of chiral amino acids and sugars during roasting or processing of the plant material. Many more are produced via pyrolytic decomposition of proteins and their reaction with sugars, sugar degradation products, or lipids and organic acids.

Over 700 volatile or partially volatile compounds have been identified in roasted coffee (Maarse and Visscher, 1989; Nishimura and Mihara, 1990). According to Feldman (1969), green coffee has no significant flavor or taste of its own. Rathbone et al. (1989) reported that (*S*)-(−)-2-(4-methoxyphenoxy)propanoic acid predominated in the aqueous extracts of roasted Colombian Arabica coffee beans. Likewise, over 500 volatile compounds have been identified in black tea (Mick and Schreier, 1984; Maarse and Visscher, 1989). The relative concentrations of these volatiles have been shown to be sensitive to the manufacturing process (Kobayashi et al., 1985) as well as to agronomic practices (Owuor et al., 1987; Baruah et al., 1986). Likewise, the processing of cocoa beans involves drying, fermentation, and roasting (Rohan, 1963). Over 500 volatile compounds have been identified in roasted cocoa beans (Kim and Keeney, 1983; Maarse and Visscher, 1989).

Recently, chiral GC stationary phases have been reported which are based on amorphous derivatized cyclodextrins (König et al., 1988; Armstrong et al., 1990b). Although native cyclodextrins have been used successfully for chiral separations by high-performance liquid chromatography (HPLC), their crystallinity made them unsuitable for GC stationary phases. However, amorphous derivatized cyclodextrins have proven to produce highly efficient enantiomeric separations. Recently, cyclodextrin-based GC columns have been used to resolve a number of components from foods and beverages (Mosandl et al., 1990a; Armstrong et al., 1990a) and essential oils (Mosandl et al., 1990b; Hener et al., 1990; König et al., 1992) and theaspiranes from a variety of fruits and berries (Schmidt et al., 1992). The purpose of this study was to determine the feasibility of enantioresolving chiral compounds likely to be found in coffee, tea, or cocoa by capillary GC using cyclodextrin-based stationary phases.

EXPERIMENTAL METHODS

Columns. Heptakis(2,6-di-*O*-pentyl-3-trifluoroacetyl)-β-cyclodextrin (B-TA) and octakis(2,6-di-*O*-pentyl-3-trifluoroacetyl)-γ-cyclodextrin (G-TA) were synthesized as reported previously (Li et al., 1990). The permethyl-*O*-[(*S*)-2-hydroxypropyl]-β-cyclodextrin (B-PH) was also made as previously reported (Armstrong et al., 1990b,c). The fused-silica capillary tubing (0.25 mm i.d.), obtained from Alltech, was coated via the static method (Bouche and Verzele, 1968). These gas chromatographic stationary phases are now commercially available through Advanced Separation Technologies, Inc. (Whippany, NJ) as the Chiraldex B-TA, Chiraldex G-TA, and Chiraldex B-PH columns.

Standards and Reagents. All chiral compounds used in this study as well as trifluoroacetic anhydride were obtained from Aldrich Chemical Co. (Milwaukee, WI) or ICN Biomedicals, Inc. (Costa Mesa, CA). The cyclodextrins were obtained from Advanced Separation Technologies, Inc. (Whippany, NJ).

Procedures. Approximately 1 mg of analyte was dissolved in 0.1 mL of ethyl ether. Trifluoroacetic anhydride (0.1 mL) was added. The solution was allowed to react for 20 min. Subsequently, anhydrous N_2 was bubbled through the solution to remove excess reagent.

Apparatus. A Shimadzu GC-8A (equipped with a flame ionization detector) gas chromatograph was used. Split injections of 0.2–0.5-μL sample size were done with a split ratio 1/100. The carrier gas was N_2.

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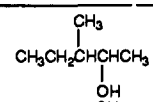
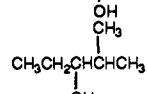
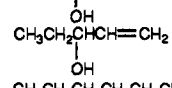
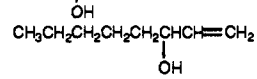
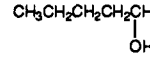
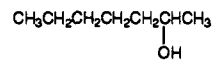
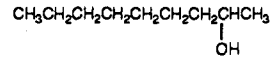
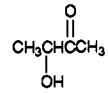
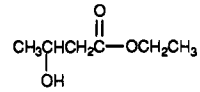
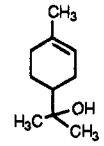
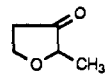
Table I. Chromatographic Data for Compounds Resolved on the B-TA Column[a]

compd	structure	T, °C	k'	α	found[b]	ref
3-methyl-2-pentanol		25	4.5[c] 6.0[c]	1.14[c] 1.15[c]	T	*d*
2-methyl-3-pentanol		30	4.85	1.05	T	*d*
1-penten-3-ol		40	1.40	1.04	T	*e, f, g, h, l, j, k*
1-octen-3-ol		50	13.15	1.03	T	*e, f, i, l*
2-hexanol		50	3.49	1.11	T	*d*
2-heptanol		60	4.27	1.09	C, CO, T	*d, i, m, n*
2-nonanol		70	10.33	1.05	T	*i*
acetoin		70	2.5	1.50	C, T	*d, i, m, o, p, q*
ethyl 3-hydroxybutyrate		50	7.60	1.13	T	*d*
α-terpineol		60	6.67	1.32	C, CO, T	*f, g, h, i, j, k, m, r*
2-methyltetrahydrofuran-3-one		60	3.82	1.06	C, T	*d, q*

[a] All separations were done on a 10 m × 0.25 mm i.d. fused silica capillary column except for 2-methyl-3-pentanol and 2-hexanol, which were analyzed on a 20-m column. All alcohols were resolved as their trifluoroacetyl derivatives. [b] Reported in coffee (C), cocoa (CO), or tea (T); see references. [c] This compound contains two chiral centers and exists as two pairs of enantiomers. The k' values are for the first and third peaks. The first α value is for the first two peaks, and the second α value is for the last two. However, it should be noted that all peaks are of about the same area and we do not know with certainty which peaks are the enantiomeric pairs and which are diastereomers. [d] Mick and Schreier, 1984. [e] Kobayashi et al., 1985. [f] Kawakami and Yamanishi, 1983. [g] Owuor et al., 1987. [h] Baruah et al., 1986. [i] Kawakami et al., 1987. [j] Tokitomo et al., 1984. [k] Takeo 1983. [l] Horita et al., 1985. [m] Spadone et al., 1990. [n] Gill et al., 1983. [o] Compton and Stout 1990. [p] Purcell and Magidman, 1984. [q] Gianturco et al., 1966. [r] van der Wal et al., 1971.

RESULTS AND DISCUSSION

Three gas chromatographic columns were found to separate the greatest number of volatile components and with the highest resolution. Tables I–III list the chromatographic data for the compounds investigated on each of these three columns. All of the compounds in this study were cited in the literature as being present in tea, coffee, and/or cocoa (see reference column in Tables I–III). Many of these components are found in a number of other food and beverage products as well. For example, acetoin and several of the chiral alcohols are found in beer and wine (Armstrong et al., 1990a). The presence of lactic acid in dairy products, limonene in citrus products, and menthol in literally hundreds of consumer products is well documented. Most of the compounds in this study are either chiral alcohols, esters, or ketones, although two of them (α-pinene and limonene) were unsaturated hydrocarbons.

Table I lists the resolution data for compounds separated on the B-TA column. As in achiral separations, retention of the chiral open-chain alcohols increased as chain length increased. Saturated alcohols seemed to have slightly better selectivity, and better enantioselectivity (α values) than unsaturated alcohols. The highest selectivity for alcohols was observed for enantiomers of 2-hexanol (α = 1.11). The highest selectivity observed on the B-TA column for any racemate was for acetoin (α = 1.50). Excellent separation (α = 1.32) was also obtained for α-terpineol. In HPLC separations using β-cyclodextrin

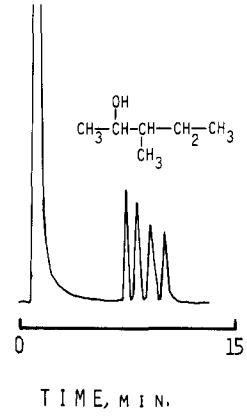
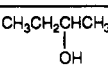
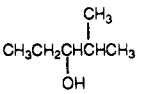
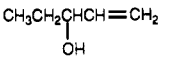
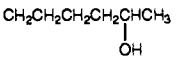
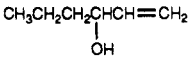
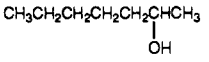
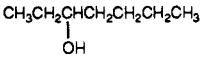
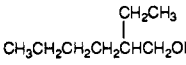
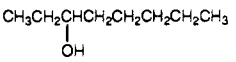
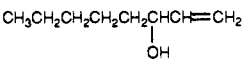
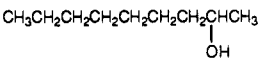
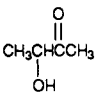
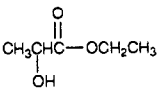
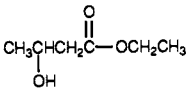
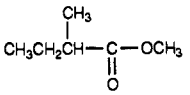
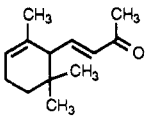
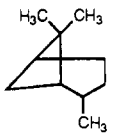


Figure 1. GC chromatogram showing the resolution of all four stereoisomers (two pairs of enantiomers) of 3-methyl-2-pentanol as the trifluoroacetyl derivative. The separation was done at 25 °C on a 10 m × 0.25 mm i.d. Chiraldex B-TA column. The split ratio was 100:1, and flame ionization detection was used.

Table II. Chromatographic Data for Compounds Resolved on the G-TA Column[a]

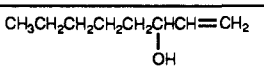
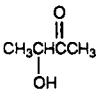
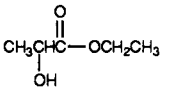
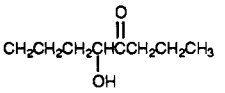
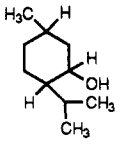
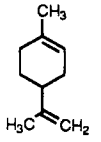
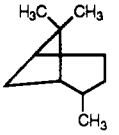
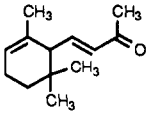
compd	structure	T, °C	k′	α	found[b]	ref
2-butanol		30	1.76	1.28	T	*h*
2-methyl-3-pentanol		40	4.76	1.33	T	*c*
1-penten-3-ol		25	8.5	1.10	T	*d, e, f, g, k, i, j*
2-hexanol		40	4.76	1.47	T	*c*
1-hexen-3-ol		30	12.40	1.27	T	*c*
2-heptanol		40	9.8	1.33	C, CO, T	*c, h, l, m*
3-heptanol		40	10.05	1.19	C	*r*
2-ethyl-1-hexanol		75	10.50	1.30	T	*h*
3-octanol		50	14.0	1.10	T	*h*
1-octen-3-ol		55	7.15	1.17	T	*d, e, h, k*
2-nonanol		60	17.93	1.11	T	*h*
acetoin		60	5.69	1.39	C, T	*c, h, n, o, p, l*
ethyl lactate		60	18.54	1.04	CO	*q*
ethyl 3-hydroxybutyrate		50	12.4	1.14	T	*c*
(±)-2-methylbutyric acid methyl ester		45	4.45	1.15	C, CO	*p, q*
α-ionone		95	3.24	2.02	T	*e, g*
α-pinene		35	10.18	1.11	T	*c*

[a] All separations were done on a 10 m × 0.25 mm i.d. fused silica capillary column except for 2-methyl-3-pentanol, (±)-2-methylbutyric acid methyl ester, ethyl lactate, and acetoin, which were analyzed on a 20-m column. All alcohols were resolved as their trifluoroacetyl derivatives. [b] Reported in coffee (C), cocoa (CO), or tea (T); see references. [c] Mick and Schreier, 1984. [d] Kobayashi et al., 1985. [e] Kawakami and Yamanishi, 1983. [f] Owuor, et al., 1987. [g] Baruah et al., 1986. [h] Kawakami et al., 1987. [i] Tokitomo et al., 1984. [j] Takeo, 1983. [k] Horita et al., 1985. [l] Spadone et al., 1990. [m] Gill et al., 1983. [n] Compton and Stout, 1990. [o] Purcell and Magidman, 1984. [p] Gianturco et al., 1966. [q] van der Wal et al., 1971. [r] Shimoda and Shibamoto, 1990.

bonded phases, it is known that having carbonyl groups adjacent to the chiral center (as in acetoin) or having the chiral center in a ring structure (as in α-terpineol) enhances chiral recognition.

Figure 1 illustrates the separation of all four isomers of trifluoroacetylated 3-methyl-2-pentanol (which has two chiral centers). Baseline resolution of both pairs of enantiomers is accomplished in less than 5 min on a 10-m Chiraldex B-TA column at 25 °C. An interesting problem arises with chiral compounds such as 3-methyl-2-pentanol that have not been adequately resolved in the past. Since no standards exist for the individual isomers, it is difficult to know the absolute configuration of the compound represented by each peak. In many cases where a compound has more than one chiral center, it is difficult to tell which peaks are enantiomers and which are diastereoisomers. Occasionally partial peak assignments can be made using GC/MS since enantiomers must have identical fragmentation patterns, while diastereomers can have slightly different patterns.

Table III. Chromatographic Data for Compounds Resolved on the B-PH Column[a]

compd	structure	T, °C	k′	α	found[b]	ref
1-octen-3-ol		25	16.84	1.03	T	*d, e, h, k*
acetoin		40	13.17	1.03	C, T	*c, h, n, o, p, l*
ethyl lactate		50	10.53	1.06	CO	*q*
butyroin		80	18.66	1.03	CO	*q*
menthol		60	7.87	1.06	CO	*q*
limonene		30	20.0	1.03	C, CO	*o, q*
α-pinene		35	8.97	1.08	T	*c*
α-ionone		95	64.40	1.04	T	*e, g*

[a] All separations were done on a 10 m × 0.25 mm i.d. fused silica capillary column except for ethyl lactate, which was analyzed on a 20-m column. All alcohols were resolved as their trifluoroacetyl derivatives. [b] Reported in coffee (C), cocoa (CO), or tea (T); see references. [c] Mick and Schreier, 1984. [d] Kobayashi et al., 1985. [e] Kawakami and Yamanishi, 1983. [g] Baruah et al., 1986. [h] Kawakami et al., 1987. [k] Horita et al., 1985. [l] Spadone et al., 1990. [n] Compton and Stout, 1990. [o] Purcell and Magidman, 1984. [p] Gianturco et al., 1966. [q] van der Wal et al.., 1971.

Table II lists the chromatographic data obtained on the Chiraldex G-TA column. Most of the chiral alcohols were better resolved on the G-TA as compared to the B-TA column (Tables I and II). In fact, more chiral compounds were resolved on the G-TA column than on any other tested chiral stationary phase. However, there are specific cases where another column shows better enantioselectivity (acetoin on the B-PH column, for example). Many times a compound will resolve only on one specific column (for example, α-terpinol and 2-methyltetrahydrofuran-3-one on B-TA or 3-heptanol, 1-hexen-3-ol, 3-octanol, and 1-octen-3-ol on G-TA). At least two things are evident from the data in Table II. First, there is not necessarily a direct correlation between retention time and enantioselectivity. In addition, relatively small structural changes within a family of molecules can have significant effects on enantioselectivity. Consider, for example, the following alcohols: 2-methyl-3-pentanol, 2-hexanol, 2-heptanol, and 3-heptanol. All were resolved at 40 °C on the same G-TA column (Table II). One of the least retained compounds, 2-hexanol, had the best enantioselectivity, while the most retained compound, 3-heptanol, had a much smaller α value. Although 2-heptanol and 3-heptanol are structural isomers (as are 2-methyl-3-pentanol and 2-hexanol), there is a significant difference in their enantioselectivity (Table II). Structure–selectivity relationships such as these are not well understood in gas chromatography. However, they are beginning to be evaluated in a systematic manner (Berthod et al., 1992).

In another study, Li (1990) reported that the heptakis-(2,6-di-*O*-pentyl-3-trifluoroacetyl)-γ-cyclodextrin (G-TA) column was the first γ-cyclodextrin-based column that was more widely useful than the analogous β-cyclodextrin-based column. The results reported here support these findings. The highest selectivities observed on the γ-cyclodextrin column were for α-ionone, 2-hexanol, and acetoin (α = 2.02, 1.47, and 1.39, respectively). The enantiomeric separation of acetoin (3-hydroxy-2-butanone) is shown in Figure 2. The large separation (α = 1.30) obtained for trifluoroacetylated 2-ethyl-1-hexanol is particularly interesting because it was obtained at 75°C and because of the lack of functionality on or immediately adjacent to the stereogenic center. It is known that chiral recognition in LC is generally enhanced for cyclic enantiomers or compounds with functional groups attached to the stereogenic center.

Table III lists the chromatographic data obtained on the permethyl-*O*-[(*S*)-2-hydroxypropyl]-β-cyclodextrin (B-PH) column. In general, the enantiomeric separations obtained on this column were of lower selectivity than those obtained on the other two columns used in this study despite the fact that lower column temperatures were used. However, the best separation for menthol (α = 1.06) was obtained on this column (see Figure 3). Menthol was not adequately separated on the other chiral stationary phases. Also, α-pinene was effectively resolved with this stationary phase (Table III).

It is likely that roasting, brewing, and other processing

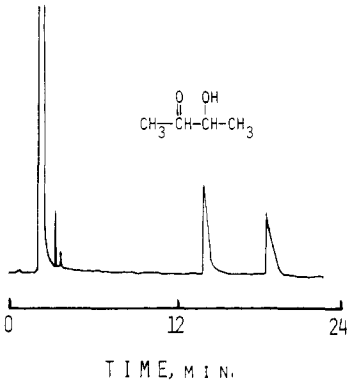


Figure 2. GC chromatogram showing the enantiomeric separation of acetoin (3-hydroxy-2-butanone). The separation was done at 60 °C on a 20 m × 0.25 mm i.d. Chiraldex G-TA column. Split ratio and detection were the same as in Figure 1.

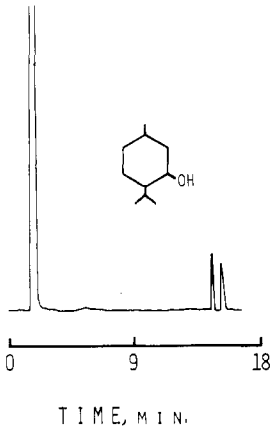


Figure 3. Gas chromatographic separation of what is reported to be (±)-menthol from Aldrich (Milwaukee, WI) as the trifluoroacetyl derivative. The separation was done at 60 °C on a 10 m × 0.25 mm Chiraldex B-PH column. Split ratio and detection were the same as in Figure 1.

procedures are involved in the production and release of the smaller chiral molecules. Enantiomeric analysis may provide a useful way to monitor these processes, thereby providing more reproducible products. Currently we are using the separations developed in this work to identify specific enantiomers and enantiomeric ratios in authentic samples of tea, coffee beans, and cocoa beans as well as other beverages and food products. Subsequently, the effect of processing on these products can be examined.

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