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Enatiomeric Separation of Branched Fatty Acids after Conversion with *trans*-2-(2,3-Anthracenedicarboximido)cyclohexanol, a Highly Sensitive Chiral Fluorescent Conversion Reagent

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(1*R*,2*R*)-2-(2,3-Anthracenedicarboximido)cyclohexanol was synthesized as a highly sensitive chiral fluorescent conversion reagent. The diastereomeric derivatives of chiral branched fatty acids that had methyl ethyl chirality from the 2 to 12 position were separated into 2 peaks by reversed-phase HPLC and detected at the 10^{-15} mole level by fluorometry.

Key words: enantiomeric separation; chiral branched fatty acid; chiral fluorescence conversion reagent; high-performance liquid chromatography

The enantiometric discrimination of both naturally occurring and synthetic materials has received much attention because the enantiomers would have different biological and toxicological properties. Therefore, determination of the absolute stereochemistry is very important for assessing these biological and toxicological properties.

High-performance liquid chromatography (HPLC) is one of the most powerful tools to separate stereoisomers. There are two modes for separating chiral compounds, one being direct and using a chiral stationary phase or a chiral mobile phase, and the other being indirect and using a chiral conversion reagent to form diastereomeric derivatives. There are some reports about chiral conversion reagents for chiral carboxylic acids.[1–5] Chiral conversion can separate stereoisomers without an expensive chiral stationary phase or mobile phase. Furthermore, it is possible to detect with high sensitivity and selectivity by using a fluorescent conversion reagent. Highly sensitive and selective detection is very advantageous for a trace analysis. However, it has been assumed that there is an intrinsic drawback in the difficulty or impossibility of separating diastereomeric derivatives having chiral centers at positions more than 4 bonds remote from each other. Therefore, it has been considerd impossible to separate branched fatty acids which had chiral centers more than three bonds remote from their carboxyl groups. In particular, the chiral discrimination of a branched alkyl chain had been thought very difficult or almost impossible by chromatographic methods and by spectrometric methods such as NMR and circular dichroism (CD).

Tanaka *et al.* reported (*S*)- and (*R*)-1-methyl-2-naphthalimidoethyl trifluoromethanesulphonate and (*S*)- and (*R*)-2-methyl-2-naphthalimidoethyl trifluoromethanesulphonate as highly sensitive chiral conversion reagents.[5] They reported the separation of such α-chiral carboxylic acids as mandelic acid. We have recently reported (*S*)-(+)-2-(2,3-anthracenedicarboximido)-1-propyl trifluoromethanesulphonate ((*S*)-2A1P-OTf) as a more sensitive chiral fluorescent conversion reagent[6,7] which has made it possible to separate the enantiomers of chiral fatty acids with a hydroxy or a methyl group at the 2, 3, 4, 5 or 6 position by HPLC.[6] This reagent has also made it possible to separate four stereoisomers of beraprost sodium, which is an analogous agent of PGI_2 consisting of two pairs of enantiomers and having chiral centers more than 7 bonds remote from the carboxyl group, by normal-phase HPLC and to detect each isomer at a fmol level.[7] An analogous reagent, (*S*)-(+)-1-(2,3-anthracenedicarboximido)-2-propanol ((*S*)-(+)-1A2P-OH), was also developed.[8] In this paper, we report the synthesis and chiral discrimination ability of a novel analogous reagent, (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanol, which has a 100% chiral *gauche* conformation.

Materials and Methods

Chemicals. (*S*)- and (*R*)-2-Amino-1-propanol and (*S*)- and (*R*)-1-amino-2-propanol were purchased from Tokyo Kasei Organic Chemicals (Tokyo, Japan). The solvents for HPLC were of HPLC grade from Kanto Chemicals (Tokyo, Japan). 2,3-Anthracenedicarboxylic anhydride was prepared by a slightly modified version of the reported method,[9] the Friedel-Crafts reaction of 1,2,4-trimethylbenzene with bonzoyl chloride using carbon disulfide as the solvent instead of methylene dichloride due to no by-products and a quantitative yield. (*S*)- and (*R*)-2A1P-OTf and (*S*)- and (*R*)-1A2P-OH were prepared by the previously reported methods.[6–8] *trans*-2-Aminocyclohexanol hydrochloride was purchased from Aldrich (Milwaukee., U.S.A.). (*S*)-Naproxen, (*S*)-2-methylbutyl *p*-toluenesulphonate, 6-bromo-1-hexene and 8-bromo-1-octene were purchased from Tokyo Kasei Organic Chemicals, the other reagents and sol-

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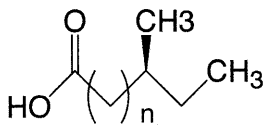
Abbreviations: 2A1P-OTf, 2-(2,3-anthracenedicarboximido)-1-propyl trifluoromethanesulphonate; 1A2P-OH, 1-(2,3-anthracene-dicarboximido)-2-propanol; CD, circular dichroism; WSC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimido hydrochloride



Fatty acid	n
(*S*)-2-methylbutyric	0
(*S*)-3-methylpentanoic	1
(*S*)-4-methylhexanoic	2
(*S*)-5-methylheptanoic	3
(*S*)-6-methyloctanoic	4
(*S*)-7-methylnonanoic	5
(*S*)-8-methyldecanoic	6
(*S*)-9-methylundecanoic	7
(*S*)-10-methyldodecanoic	8
(*S*)-11-methyltridecanoic	9
(*S*)-12-methyltetradecanoic	10

Fig. 1. Structures of the Branched Fatty Acids.

vents being purchased from Wako Pure Chemicals (Osaka, Japan).

The chiral branched fatty acids that were tested are shown in Fig. 1. (*S*)-2-Methylbutyric acid, (*S*)-3-methylpentanoic acid, (*S*)-4-methylhexanoic acid, (*S*)-5-methylheptanoic acid and (*S*)-6-methyloctanoic acid were each prepared by oxidation of the corresponding optically pure (*S*)-alcohols (Tokyo Kasei Organic Chemicals) with $KMnO_4$ in aqueous pyridine at room temperature for 12 h. Their structures were confirmed by high-resolution mass spectra (HRMS; Table 1) and ^{1}H-NMR (data are not shown) after conversion with (*S*)- and (*R*)-2A1P-OTf. Optically pure (*S*)-7-methylnonanoic acid and (*S*)-8-methyldecanoic acid were prepared by the reaction of 1-pentenyl-5-magnesium bromide with (*S*)-3-methylpentyl *p*-toluenesulphonate or (*S*)-4-methylhexyl *p*-toluene-sulphonate in the presence of CuI, respectively, this being followed by oxidative cleavage of their double bonds to carboxylic acids with $KMnO_4$ in water/dichloromethane in the presence of tetra-butylammonium bromide. Each was purified by passing through a silica gel column, eluting with n-hexane/ethyl acetate. Optically pure (*S*)-9-methylundecanoic acid and (*S*)-10-methyldodecanoic acid were prepared from 1-octenyl-8-magnesium bromide and (*S*)-2-methylbutyl or (*S*)-3-methylpentyl *p*-toluenesulphonate, respectively, and (*S*)-11-methyltridecanoic acid and (*S*)-12-methyltetradecanoic acid were prepared from 9-octadecenyl-1-magnesium chloride and (*S*)-2-methylbutyl or (*S*)-3-methylpentyl *p*-toluenesulphonate, respectively, in the same manner as that just described. Their structures were also confirmed by HRMS (Table 1) and ^{1}H-NMR (data are not shown) after conversion with (*S*)- and (*R*)-2A1P-OTf.

Table 1. HRMS Data for the (*S*) and (*R*)-2A1P Derivatives of Branched Fatty Acids

Fatty acid	Config. of* reagent	[M$^+$]	
		Calcd.	Found
(*S*)-2-methylbutyrate	*S*	$C_{24}H_{23}O_4N$	389.1610
	R	389.1627	389.1638
(*S*)-3-methylpentanoate	*S*	$C_{25}H_{25}O_4N$	403.1765
	R	403.1783	403.1795
(*S*)-4-methylhexanoate	*S*	$C_{26}H_{27}O_4N$	417.1927
	R	417.1940	417.1933
(*S*)-5-methylheptanoate	*S*	$C_{27}H_{29}O_4N$	431.2089
	R	431.2096	431.2093
(*S*)-6-methyloctanoate	*S*	$C_{28}H_{31}O_4N$	445.2257
	R	445.2253	445.2238
(*S*)-7-methylnonanoate	*S*	$C_{29}H_{33}O_4N$	459.2409
	R	459.2410	459.2409
(*S*)-8-methyldecanoate	*S*	$C_{30}H_{35}O_4N$	473.2565
	R	473.2566	473.2565
(*S*)-9-methylundecanoate	*S*	$C_{31}H_{37}O_4N$	487.2722
	R	487.2723	487.2726
(*S*)-10-methyldodecanoate	*S*	$C_{32}H_{39}O_4N$	501.2875
	R	501.2879	501.2885
(*S*)-11-methyltridecanoate	*S*	$C_{33}H_{41}O_4N$	515.3035
	R	515.3036	515.3032
(*S*)-12-methyltetradecanoate	*S*	$C_{34}H_{43}O_4N$	529.3193
	R	529.3192	529.3194

* Configuration of the 2A1P-OTf reagent.

Preparation of trans-2-(2,3-anthracenedicarboximido)cyclohexanol. 2,3-Anthracenedicarboxylic anhydride (1.0 g) was dissolved in 350 ml of toluene by refluxing, and *trans*-2-aminohexanol hydrochloride (0.8 g), DMF (100 ml) and triethylamine (30 ml) were added while refluxing. After refluxing for 10 h, 300 ml of the solvent was removed through a Dean-Stark tube. After cooling, 500 ml of ethyl acetate was added, and the solution was successively washed with 0.2 N NaOH, 0.2 N HCl and saturated $NaHCO_3$. The solution was dried over magnesium sulfate, the solvent being evaporated under reduced pressure. *trans*-2-(2,3-Anthracenedicarboximido)cyclohexanol was obtained as a yellow crystal from ethylacetate (1.31 g, 93.7%), mp >300°C. HRMS (FAB.) [M+1]$^+$: calcd. for $C_{22}H_{20}NO_3$, 346.1443; found, 346.1449.

Preparation of trans-(1R,2R)-(2,3-anthracenedicarboximido)cyclohexanol. Method A, involving kinetic resolution by esterification with (*S*)-naproxen: *trans*-2-(2,3-Anthracenedicarboximido)cyclohexanol (2 g, 5.8 mmol), 4-(dimethylamino)pyridine (0.5 g) and pyridine (5 ml) were dissolved in a 1000 ml mixture of toluene/acetonitrile (1:1) at 0°C, and then (*S*)-(+)-naproxen (6.0 g, 26 mmol) and dicyclohexylcarbodiimide (6.2 g) were added. After stirring for 2.5 h at 0°C, the reaction mixture was extracted with toluene/ethyl acetate and then successively washed with 0.2 N HCl, 0.2 N NaOH and saturated $NaHCO_3$. After drying with magnesium sulfate, three spots were observed by a silica gel TLC developed with toluene: ethyl acetate (6:1, v/v) at R_f 0.21, 0.64 and 0.68. The compound with R_f 0.21 was isolated in a silica gel column (400 g) developed with toluene/ethyl acetate (6:1, v/v) and recrystallized from toluene/ethyl acetate. *trans*-(1*R*,2*R*)-2-(2,3-Anthracenedicarboximido)cyclohexanol was obtained as a yellow crystal (315 mg, 15.8%), mp >300°C. HRMS (FAB.) [M+1]$^+$: calcd. for $C_{22}H_{20}NO_3$, 346.1443; found, 346.1443. $[\alpha]_D^{20}$ −39.0° (*c*=0.212, DMF).

The optical purity of the reagent was more than 99.6% e.e., this being determined by reversed-phase HPLC after converting with optically pure (*S*)-4-methylhexanoic acid. The absolute configuration was (1*R*,2*R*) as determined by the exciton CD method after converting with 4-methoxycinnamic acid, mp 218°C. HRMS

(FAB) $[M+1]^+$: calcd. for $C_{32}H_{28}NO_5$, 506.4967; found, 506.4972; $[\alpha]_D^{20}$ $-436°$(c=0.128, DMF); CD(ethyl acetate) [Θ] (nm): -2.955×10^5 (311.5), 2.013×10^5 (276.5). Method B: Both optically pure (1*S*,2*S*)- and (1*R*,2*R*)-*trans*-2-aminocyclohexanol were prepared from 1,2-epoxycyclohexane and (*R*)-phenylethylamine by a 2-step reaction.[10] Each optically pure *trans*-2-aminocyclohexanol was reacted with 2,3-anthracenedicarboxylic anhydride to form *trans*-2-(2,3-anthracenedicarboxyimido)cyclohexanol by the method already described.

Sample preparation procedure. The branched fatty acids were esterified with the reagents by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimido hydrochloride (WSC) in the presence of 4-(di-methylamino)pyridine in toluene and acetonitrile (1:1, v/v). To the solution of a fatty acid, an excess amount (more than 3 equimolar) of the reagent and a catalytic amount of 4-(dimethylamino) pyridine were added. After adding an excess amount of WSC, the mixture was reacted at r.t. for more than 10 h. An aliquot from the reaction was loaded onto a silica gel TLC plate (10 cm length, silica gel 60 F_{254}; Art-5744, Merck, Darmstadt, Germany) and developed with toluene/ethyl acetate (5:1, v/v). The target spot was collected and packed into a pasteur pipette, which was eluted with ethyl acetate/ethanol (4:1, v/v). After evaporating the solvent, the residue was dissolved in methanol and used for an HPLC analysis.

Equipment. The HPLC pump used was a Jasco PU-980 type (Japan Spectroscopic Co., Tokyo, Japan) equipped with a Rheodyne 7125 sample injector fitted with a 20-μl sample loop. The fluorescence detector was a Jasco FP-920 unit with a 3-μl flow cell, and the integrator was a Chromatocorder 12 (System Instrument, Tokyo, Japan). Cryocool CC100-II was used to control the column temperature. High-resolution mass spectra were measured in the EI mode or FAB mode by a JMS HX-105 instrument (Jeol, Tokyo, Japan), using 4-nitrobenzaldehyde as a matrix. ^{1}H-NMR spectra were measured in $CDCl_3$ by Varian Gemini 2000/300 and/or Varian Unity Inova 500 instruments.

HPLC separation. The derivatives of the branched fatty acids were separated in an ODS column (Develosil ODS-3, 3 μm, 4.6 mm i.d. × 150 mm; Nomura Chemical Co., Aichi, Japan) at 0.6 ml/min. Detection was carried out by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm). A mixed solution of methanol/acetonitrile/water or methanol/acetonitrile/n-hexane was used as the mobile phase, and the column temperature was kept between $-50°$C and 24°C. The optimized conditions for each derivative are shown in the footnote to Table 2.

Results and Discussion

Designation and synthesis of (1R,2R)-2-(2,3-anthracendicarboximido)cyclohexanol

In both 2A1P-OTf and 1A2P-OH, the 2,3-anthracenedicarboximide group played important roles not only for sensitive detection as a strong fluorophore but also for inducing of intramolecular CH-π interaction. The *gauche-trans* conformation of the chiral ethanolamine moiety is the most stable one because of the *gauche* effect and the CH-π interaction, since the *gauche-trans* conformers themselves should have a clockwise (for the *S* configuration) or counter-clockwise (for the *R* configuration) orientation and bring the chiral center of a branched fatty acid close to the aromatic ring. Therefore, it was expected that superior chiral recognition ability would be obtained by fixing the conformation around the ethanolamine part. We designed the analogous chiral conversion reagents (Fig. 2), (1*S*,2*S*)- and (1*R*,2*R*)-2-(2,3-anthracendicarboximido)cyclohexanol, that have a 100% chiral *gauche* conformation.

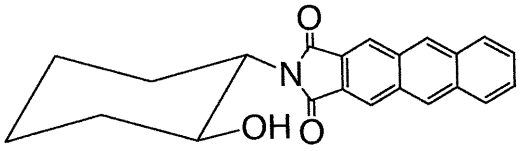


Fig. 2. *trans*-2-(2,3-Anthracenedicarboximido)cyclohexanol.

At first, racemic *trans*-2-(2,3-anthracenedicarboximido)cyclohexanol was prepared from commercially available racemic *trans*-2-aminocyclohexanol hydrochloride and 2,3-anthracenedicarboxylic anhydride in a similar manner to that reported previously. It was possible to obtain an optically pure (1*R*,2*R*) reagent by kinetic resolution. In the reaction, the (1*S*,2*S*) reagent reacted with (*S*)-(+)-naproxen much faster than the (1*R*,2*R*) isomer. It was possible to obtain optically pure (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexanol and the diastereomeric naproxen esters by stopping the reaction before its completion. The unreacted reagent showed more than 99.6% e.e. and a yield of 15.8%. The two diastereomeric derivatives could be separated in a silica gel column, and each of them was hydrolyzed by refluxing in aqueous methanol with a catalytic amount of sulfuric acid for 1 week. These procedures enabled the (1*R*,2*R*) reagent to be obtained with more than 98.5% e.e., while the (1*S*,2*S*) isomer had lower optical purity (75% e.e.), probably due to the racemization of naproxen during the reaction. Their absolute configurations were determined by CD spectral mesurements after conversion to 4-methoxycinnamate. The unreacted reagent showed a typical exciton CD spectrum with a negative first Cotton effect. The optical purity of each was determined by reversed-phase HPLC after conversion with (*S*)-4-methylhexanaoic acid. Each optically pure *trans*-2-(2,3-anthracenedicarboximido)cyclohexanol was also obtained from the corresponding optically pure *trans*-2-aminocyclohexanol that had been prepared by the reported method.[10]

Conformational analysis by ^{1}H-NMR

Figure 3 shows the different chemical shifts of the terminal methyl protons between the methyl and *trans*-2-(2,3-anthracenedicarboximido)cyclohexyl esters of straight-chain saturated fatty acids (C2:0–C18:0). The

terminal methyl protons of the *trans*-2-(2,3-anthracenedicarboximido)cyclohexyl esters were shifted to a higher field than those of the corresponding methyl esters. The shift difference reached a maximum for the pentanoate ($\Delta\delta$=0.394 ppm) and then gradually decreased, and almost no difference was apparent with the myristate (C14:0). While this pattern is similar to those of the 2A1P- and 1A2P-derivatives, the shifts of the *trans*-2-(2,3-anthracenedicarboximido)cyclohexyl esters were about 2.5 times larger than those of the acyclic reagents.

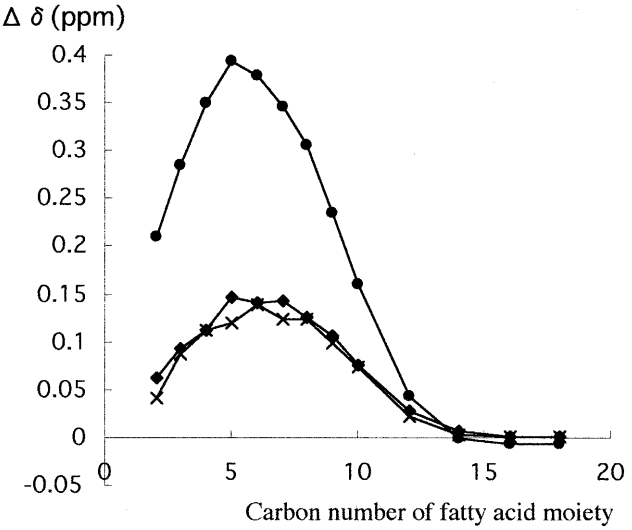


Fig. 3. Effects of Conversion Reagents on the ^{1}H-NMR Chemical Shifts of Terminal Methyl Protons in Straight Chain Saturated Fatty Acids.

Difference are shown between the terminal methyl proton chemical shifts ($\Delta\delta$) of the methyl esters and the corresponding esters of *trans*-2-(2,3-anthracenedicarboximido)cyclohexanol (●), 2A1P-OH (◆) and 1A2P-OH (×) measured in $CDCl_3$ at 20°C.

In the ^{1}H-NMR spectrum of the stearic acid derivative, some methylene protons of the alkyl chains were shifted to a higher field by up to 0.5 ppm than those of methyl stearate, the shift also being much larger than those of the derivatives of acyclic reagents that have previously been reported.[8] These results mean that the alkyl chain of the ester was located over the anthracenedicaroximide ring more rigidly than in the 2A1P- and 1A2P-derivatives by fixing to one 100% *gauche* comformer, and this also led us to expect the formation of stronger CH-π interaction between the alkyl chain of the ester and an aromatic imide ring. This was evident from the fact that only the cyclic reagent could discriminate the chiral branched methyl group at the 11 position by ^{1}H-NMR. The branched methyl group of the (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexyl ester of (*S*)-11-methyltridecanoic acid appeared at 0.769 ppm, and that of the (1*S*,2*S*) isomer was at 0.772 ppm in $CDCl_3$ at −20°C, while those of both the (*R*)- and (*S*)-2A1P-esters appeared at 0.792 ppm under the same conditions.

Separation of the branched acid derivatives by reversed-phase HPLC

The separation of some enatiomeric branched fatty acid esters of (1*R*,2*R*)-2-(2,3-anthracenedicarboximido) cyclohexanol was tested by using an ODS column. The derivatives generally showed larger α values (selectivity factors) by eluting with a methanol solution than with an acetonitrile solution, while the peak resolution (Rs value) was not improved by eluting with methanol because of peak broadening. A mixture of methanol and acetonitrile (3:2, v/v) was thus used as the main solvent for the mobile phase. The elution power of the mobile phase was controlled by adding water or n-hexane to the main solvent. Figure 4 shows the effects of column temperature on the α and Rs values. The optimum tempera-

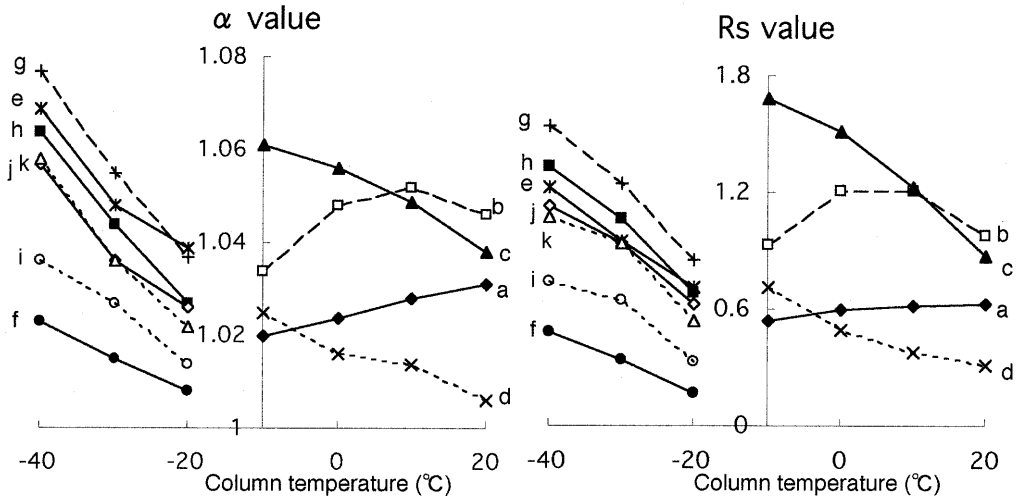


Fig. 4. Effects of Column Temperature on the Enatiomeric Separation of *trans*-2-(2,3-Anthracenedicarboximido)cyclohexyl Esters.

The samples were 2-methylbutyrate (a, ◆), 3-methylpentanoate (b, □), 4-Methylhexanoate (c, ▲), 5-methylheptanoate (d, ×), 6-methyloctanoate (e, ✳), 7-methylnonanoate (f, ●), 8-methyldecanoate (g, +), 9-methylundecanoate (h, ■), 10-methyldodecanoate (i, ○), 11-methyltridecanoate (j, ◇) and 12-methyltetradecanoate (k, △). The column used was a Develosil ODS-3 (4.6 × 150 mm) and the flow rate was 0.6 ml/min. The mobile phases were methanol/acetonitrile/water (300:200:70 for a–d), and methanol/acetonitrile/n-hexane (300:200:10 for e–h and 300:200:20 for i–k).

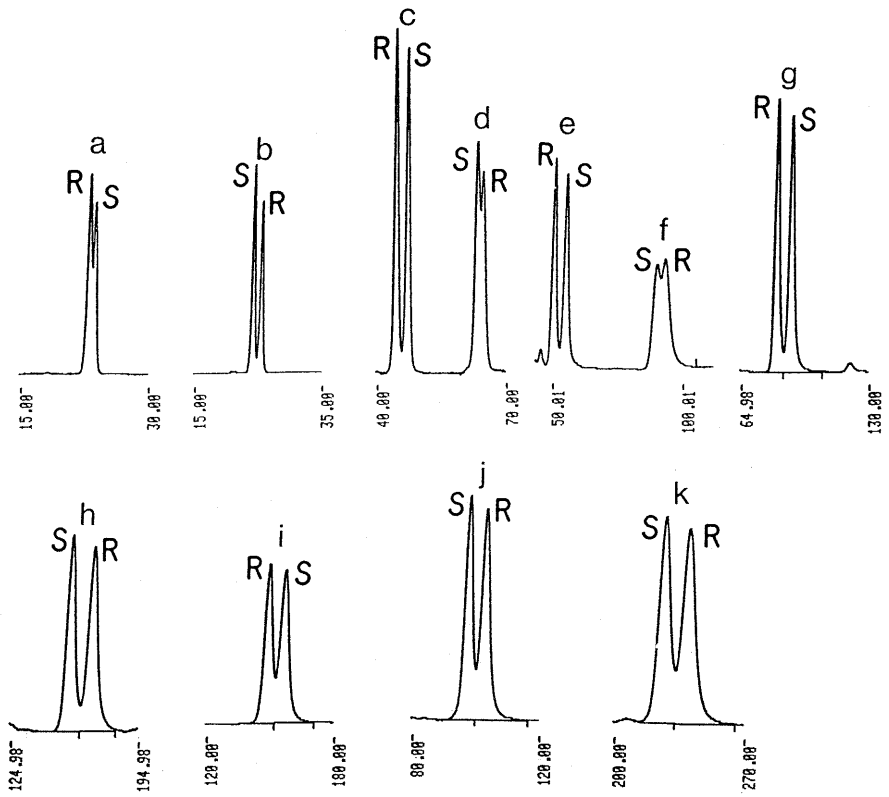


Fig. 5. Typical Chromatograms of the (1R,2R)-2-(2,3-Anthracenedicarboximido)cyclohexyl Derivatives of Branched Fatty Acids.
The amples were 2-methylbutyrate (a), 3-methylpentanoate (b), 4-methylhexanoate (c), 5-methylheptanoate (d), 6-methyloctanoate (e), 7-methylnonanoate (f), 8-Methyldecanoate (g), 9-methylundecanoate (h), 10-methyldodecanoate (i), 11-methyltridecanoate (j) and 12-methyltetradecanoate (k). The HPLC conditions are as shown in Table 2.

ture for resolution decreased as the branched methyl group moved further away from the carboxyl groups. The increase of α value by methanol might be partly attributed to fixation of the conformation by increasing the viscosity of the mobile phase. A low column temperature was also effective for fixation of the conformation by both the low temperature itself and the increase in viscosity. However, the increase in viscosity reduced the column efficiency, and this might have caused the peak broadening. Improvement of the chiral discrimination by similar means was also apparent with ^{1}H-NMR. Both the degree of the higher field shift of some methylene protons and the different chemical shifts between the branched methyl groups were larger in dimethylsulfoxide-d_6 than in $CDCl_3$ at 20°C. In $CDCl_3$, a lower temperature also induced similar effects.

Figure 5 and Table 2 show typical chromatograms and chromatographic data, respectively, under the optimized conditions. All derivatives could be separated into two peaks by the proposed method. This allowed us to discriminate the chirality consisting of a methyl group and an ethyl group from the 2 position to at least the 12 position, which has been considered the most difficult case in enatiomeric discrimination because of the small differences.

Although, for some branched fatty acids such as 2-methylbutyric acid and 7-methylnonanoic acid, 1A2P-OH showed superior enantiomeric separation ability to that of the proposed reagent (Table 3), *trans*-2-(2,3-anthracenedicarboximido)cyclohexanol was the only reagent which could separate all the enatiomeric branched

Table 2. Chromatographic Data for the *trans*-(1*R*,2*R*)2-(2,3-Anthracenedicarboximido)-cyclohexyl Derivatives of Some Branched Fatty Acids

Fatty acid	k′		α	Rs	Conditions
	S	*R*			
2-methylbutyrate	9.29	9.00	1.032	0.812	1
3-methylpentanoate	9.56	10.12	1.058	1.565	2
4-methylhexanoate	19.21	18.11	1.061	1.687	3
5-methylheptanoate	26.08	26.72	1.025	0.714	3
6-methyloctanoate	21.73	19.81	1.097	1.530	4
7-methylnonanoate	35.73	37.01	1.036	0.570	4
8-methyldecanoate	38.54	35.33	1.091	1.635	5
9-methylundecanoate	67.43	72.58	1.076	1.375	5
10-methyldodecanoate	67.03	63.76	1.051	1.032	6
11-methyltridecanoate	41.23	43.57	1.057	1.130	7
12-methyltetradecanoate	97.09	102.7	1.058	1.080	7

Conditions: The mobile phases and column temperatures were 1) methanol/acetonitrile/water (v/v/v)=300/200/120 at 24°C, 2) methanol/acetonitrile/water=300/200/90 at 10°C, 3) methanol/acetonitrile/water=300/200/70 at −10°C, 4) methanol/acetonitrile/n-hexane=300/200/5 at −50°C, 5) methanol/acetonitrile/n-hexane=300/200/7.5 at −40°C, 6) methanol/acetonitrile/n-hexane=300/200/10 at −40°C, and 7) methanol/acetonitrile/n-hexane=300/200/20 at −40°C. The other conditions are as described in the Materials and Methods section.

Table 3. Chromatographic Data for the (*S*)-1-(2,3-Anthracenedicarboximido)-2-propyl Derivatives of Some Branched Fatty Acids

Fatty acid	k′		α	Rs	Conditions
	S	*R*			
2-methylbutyrate	10.36	10.86	1.048	1.356	8
3-methylpentanoate	14.63	13.85	1.056	1.687	8
4-methylhexanoate	19.04	20.41	1.072	2.227	8
5-methylheptanoate	25.12	24.49	1.026	0.524	9
6-methyloctanoate	11.64	12.54	1.077	1.470	10
7-methylnonanoate	18.51	17.66	1.048	0.856	10
8-methyldecanoate	24.79	27.64	1.120	2.092	10
9-methylundecanoate	49.37	44.66	1.105	1.872	10
10-methyldodecanoate	33.71	36.41	1.080	1.256	11
11-methyltridecanoate	46.00	43.75	1.051	1.020	12
12-methyltetradecanoate	56.95	56.78	1.003	0.053	13

Conditions: The mobile phases and column temperatures were 8) methanol/acetonitrile/water (v/v/v)=250/160/90 at 0°C, 9) methanol/acetonitrile/water=300/200/30 at −30°C, 10) methanol/acetonitrile/n-hexane=300/200/5 at −40°C, 11) methanolo/acetonitrile/n-hexane=300/200/10 at −40°C, 12) methanol/acetonitrile/n-hexane=300/200/15 at −40°C, and 13) methanol/acetonitrile/n-hexane=300/200/20 at −40°C. The other conditions are as described in the Materials and Methods section.

fatty acids that were tested, including 5-methylheptanoic acid and 12-methyltetradecanoic acid, which could not be separated by conversion with 1A2P-OH. This allowed us to use these reagents complementarily to get better enantiomeric discrimination.

It should be noted that, up to 11-methyltridecanoate by conversion with the (1*R*,2*R*)-reagent, all (*S*)-branched fatty acid esters with a methyl group at the odd-number carbon were eluted faster than the corresponding (*R*)-branched fatty acid esters, wheil (*R*)-branched fatty acid esters with a methyl group at an even-number position were eluted faster than the corresponding (*S*)-branched fatty acid esters, although the order changed with the 12-methyltetradecanoate. Since the main alkyl chains of the acids would have a straight zig-zag conformation over the aromatic ring according to their ^{1}H-NMR data, the branched methyl groups at odd-number carbons of the (*S*)-branched fatty acid esters have the same geometry against the aromatic ring, while those of the corresponding (*R*)-branched fatty acids have a different geometry. The elution order could be explained by this geometric difference.

Since the 2,3-anthracenedicarboximide group is a very strong fluorophore, it was possible to detect the derivatives with very high sensitivity. As a representative case, the detection limit for (1*R*,2*R*)-2-(2,3-anthracenedicarboximido)cyclohexyl (*S*)-8-methyldecanoate was 9 fmol (signal-to-noise ratio=3) under the optimized conditions. Since there was no significant difference in the fluorescence intensity among the fatty acid derivatives, it is possible to determine the absolute configuration of branched fatty acids at femto mole levels by comparing with standard samples.

Conclusion

We developed a novel highly sensitive chiral conversion reagent which makes it possible to discriminate the chirality of branched fatty acids at least up to the 12 position by reversed-phase HPLC and fluorometric detection. The enantiomeric discrimination of a methyl and ethyl will be more difficult than that of a methyl and propyl or larger alkyl group because of the smaller difference around the chiral center. Therefore, it will be possible to discriminate the chirality of longer-chain branched fatty acids than those tested here. This conversion method is also applicable to discriminate remote chirality by ^{1}H-NMR.

Branched fatty acids have been found in some mammalian tissues,[11] fish[12] and starfish.[13] A new ceramide with a novel branched fatty acid has recently been isolated from the epiphytic dinoflagellate, *Coolia monotis*.[14] That branched fatty acid moiety, Z-(2*R*)-2-hydroxy-15-methyloctadecanoic acid, should be chemically converted into 12-methylpentadecanoic acid. Some sphingoglycolipids which have a branched methyl group at the 15 position of their sphingosine moieties have also been isolated from echinoderms by K. Yamada *et al.* These sphingosines can be chemically converted into 11-methyltetradecanoic acid. After this conversion, it will be possible to determine their absolute configuration by the proposed method. Highly sensitive remote chiral discrimination methods will become one of the most powerful tools for the stereochemical determination of many naturally occurring materials.

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