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Enantiomeric Separation of Carboxylic Acids Having Chiral Centers Remote from the Carboxyl Group by Labelling with a Chiral Fluorescent Derivatization Reagent

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Abstract: Enantiomeric separations of 2-, 3-, 4-, 5- and 6-methyl fatty acids and 3-, 4- and 5-hydroxy fatty acids derivatized with (*S*)-(+)-2-(anthracene-2,3-dicarboximido)-1-propyl trifluoromethanesulfonate are described. Although there are 4-8 bond distances between the chiral centers of these diastereomeric derivatives, they are separated on HPLC and detected at fmol levels. © 1997 Elsevier Science Ltd.

The chiral derivatization method, an indirect optical resolution method, is one of the most widely used methods in chiral analysis as well as the direct separation method using a chiral stationary phase or a chiral mobile phase. Although many chiral derivatization reagents have been reported, the difficulty, or impossibility, of separating the diastereomeric derivatives having chiral centers that are remote by more than 4 bonds has been thought to be an intrinsic drawback of the methodology.[1] Recently, we developed a highly sensitive fluorescent derivatization reagent, 2-(anthracene-2,3-dicarboximido)ethyl trifluoromethanesulfonate (AE-OTf), for carboxylic acids.[2] By labelling with the reagent, it was possible to separate 18 kinds of long-chain fatty acids, including poly unsaturated fatty acids, within 32 min on a reversed-phase HPLC with an isocratic elution mode. The reagent was successfully applied to the determination of DSP toxins.[3,4] By this method, the derivatives were detected at sub-fmol levels. [2-4]

The ^{1}H-NMR studies of some AE-O-esters of long-chain fatty acids indicate that these compounds take the bent conformation as shown in Fig.1-a. Since some proton signals of the hydrocarbon chain were shifted to higher field, those protons would be located over the anthracene ring. Especially, the protons at the β and γ positions of the ester carbonyl group are shifted about 0.06 and 0.07 ppm, respectively, to higher field than those of the corresponding methyl ester, which indicated that these protons located over the anthracene ring and the bending took place at the amino ethanol moiety of the reagent. The high separation potency of AE-O-esters on an HPLC could be ascribed to the ability to form the bent conformation which was formed by the preferred gauche comformation at the amino ethanol moiety and intramolecular CH- π interaction. Therefore, it can be expected that the diastereomeric derivatives of chiral carboxylic acids having chiral centers at a remote position from their carboxyl groups labelled with a chiral AE-OTf analogue should form the bent conformation, and each bent conformer of the diastereomeric derivatives would have similar property to those of *cis* and *trans* isomers of cyclic compounds to induce chiral recognition (Fig.2).

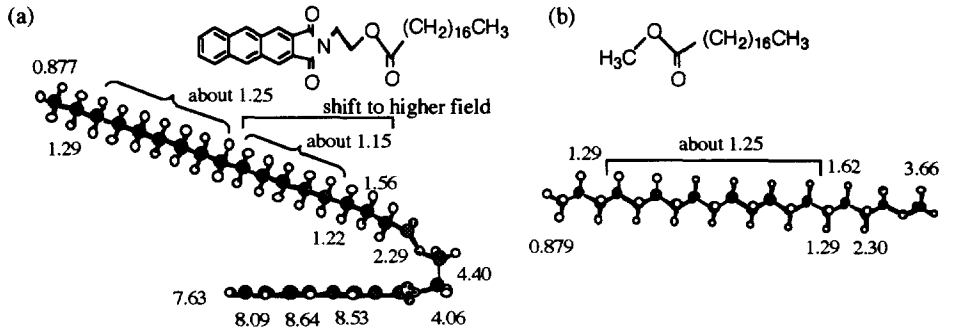


Fig.1. One possible comformer of AE-O-stearate (a) and ^{1}H-chemical shifts (ppm; 500mHz, $CDCl_3$)
(a) AE-O-stearate, (b) methyl stearate

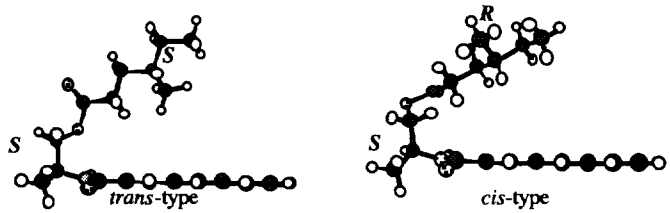
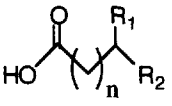


Fig.2. Possible comformers of (*S*) and (*R*)-4-methylhexanoate derivatized with (*S*)-AP-OTf

In this communication, we report the preparation of a chiral AE-OTf analogue and the results of the HPLC separation of diastereomeric derivatives of some branched fatty acids **1-8** and mono hydroxy fatty acids **9-11** (Fig.3) with the chiral reagent. The optically pure (*S*)-(+)-2-(anthracene-2,3-dicarboximido)-1-propyl trifluoromethanesulfonate [(*S*)-**12**] [5] were prepared by a similar method to that for AE-OTf with commercially available optically pure (*S*)-2-amino-1-propanol [6] instead of 2-aminoethanol for AE-OTf (Scheme 1). Here we also prepared (*R*)-**12** by using (*R*)-2-amino-1-propanol to elucidate the resolutions of the diastereomeric derivatives. The derivatizations of carboxylic acids with the reagent were performed by slightly modified conditions of a previously described method [2-4], that is, they were performed in dry acetonitrile at room temperature for more than 30 min in the presence of 1.5 equivalent of tetraethyl ammonium carbonate (TEAC). The resultant AP-O-esters were purified with a silicagel thin layer chromatography developed with toluene/ethyl acetate before HPLC analysis.

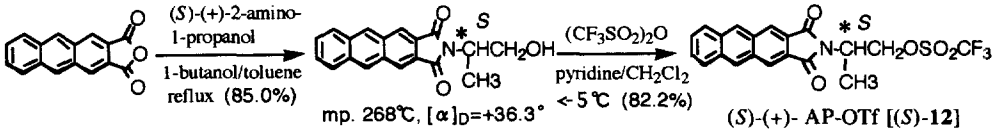


Acids	1	2	3	4	5	6	7	8	9	10	11
n =	0	1	2	3	4	0	2	2	1	2	3
R_1 =	Me	Me	Me	Me	Me	Me	Me	Me	OH	OH	OH
R_2 =	Et	Et	Et	Et	Et	Pro	Bu	Pen	Et	Hex	Pen
Configuration	*S*	*S*	*S*	*S*	*S*	*rac.*	*rac.*	*rac.*	*R*	*R*	*R*

Me = methyl, Et = ethyl, Pro = n-propyl, Bu = n-butyl, Pen = n-pentyl, Hex = n-hexyl

Fig.3. Structures of branched fatty acids (**1-8**) and mono-hydroxy fatty acids (**9-11**)

Scheme 1: Synthesis of (*S*)-(+)- AP-OTf



The optically active branched fatty acids tested were (*S*)-2-methylbutanoic acid (**1**), (*S*)-3-methylpentanoic acid (**2**), (*S*)-4-methylhexanoic acid (**3**), (*S*)-5-methylheptanoic acid (**4**) and (*S*)-6-methyloctanoic acid (**5**) which were prepared by oxidation of the corresponding (*S*)-alcohols with $KMnO_4$. Racemic 2-methylpentanoic acid (**6**), 4-methyloctanoic acid (**7**) and 4-methylnonanoic acid (**8**) were also tested. Figure 4 shows typical chromatograms of AP-derivatives on an ODS column (Develosil ODS UG-3, 4.6 mm i.d. X 150 mm) eluted with acetonitrile / water at 1.0 ml/min and 0℃. Here, the detection was carried out by monitoring of the fluorescence intensities at 462 nm (exciation at 298 nm). Although the diastereomeric derivatives of **1** and **4** were not separated into 2 peaks, the diastereomeric isomers of **1** and **4** had different retention times and they showed Rs values of 0.45 and 0.21, respectively, as shown in Table 1. It should be noted that the longer-chain fatty acids gave much better resolution than the corresponding shorter-chain ones and that the derivatives of (*S*)-fatty acids which had a chiral center at an even number carbon eluted faster than (*R*)-fatty acids while the derivatives of (*S*)-fatty acids which had a chiral center at an odd number carbon eluted slower. These results suggested that the reagent could recognize the chirality of methyl and ethyl groups at a remote position from the tagged carbonyl group and much better separation could be obtained in longer-chain fatty acids which had a methyl group at 2, 3, 4, 5 or 6 position.

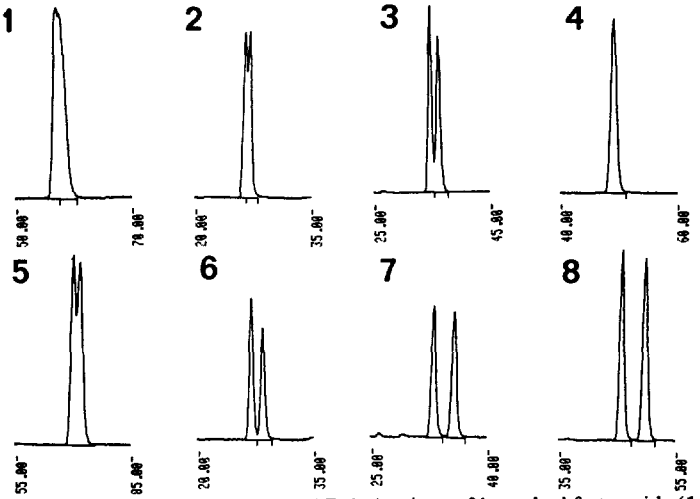


Fig.4. Typical chromatograms of (*S*)-AP derivatives of branched fatty acids (**1-8**) The (*S*)-AP derivative of **1** was eluted with acetonitrile/water (60:40, v/v), and those of **7** and **8** were eluted with acetonitrile/water (85:15, v/v). Other derivatives were eluted with acetonitrile/water (75:25, v/v).

Table 1. Diastereomeric resolution of (*S*)-AP derivatives of branched fatty acids on a reversed-phase column

Fatty acids	Mobile phase	r.t.(min) / *k'*		α	Rs
1	a	57.17 / 34.01 (*S*)	58.22 / 34.65 (*R*)	1.019	0.453
2	b	26.48 / 15.94 (*R*)	27.01 / 16.28 (*S*)	1.021	0.553
3	b	34.77 / 21.25 (*S*)	36.22 / 22.17 (*R*)	1.043	2.62
4	b	50.99 / 31.62 (*R*)	51.40 / 31.89 (*S*)	1.009	0.211
5	b	70.50 / 44.11 (*S*)	72.26 / 45.23 (*R*)	1.025	0.726
6	b	26.89 / 16.20	28.44 / 17.20	1.062	1.60
7	c	33.05 / 20.15	35.67 / 21.82	1.083	1.182
8	c	45.74 / 28.26	49.74 / 30.82	1.091	2.41

Mobile phase; acetonitrile/ water (v/v) = 60/40 (a), 75/25 (b) and 85/25 (c)

The optically active hydroxy fatty acids were also tested, (*R*)- and (*S*)-3-hydroxybutanoic acids [(*R*)-**9** and (*S*)-**9**], (*R*)-4-hydroxydecanoic acids [(*R*)-**10**] and (*R*)-5-hydroxydecanoic acid [(*R*)-**11**]. Here, the hydroxy acids (*R*)-**10** and (*R*)-**11** were prepared by hydrolysis of (*R*)-γ-decanolactone and (*R*)-δ-decanolactone with tetraethylammonium hydroxide, respectively. After complete evaporation of the solvent, they were successively derivatized with AP-OTf without addition of TEAC.

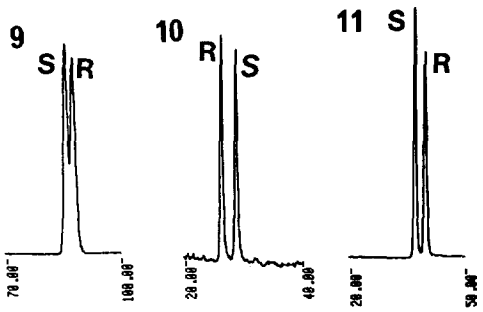


Fig.5. Typical chromatograms of (*S*)-AP derivatives of hydroxy fatty acids (**9-11**)
The Rs values of (*S*)-AP derivatives of **9**, **10** and **11** were 0.876, 2.88 and 2.43, respectively.

Figure 5 shows typical chromatograms of the diastereomeric derivatives of the hydroxy fatty acids **9-11** labelled with (*S*)-AP-OTf. They were separated on a Develosil 60-3 (4.6 mm i.d. x 250 mm) eluted with water saturated n-hexane / ethyl acetate (41:9, v/v; for **9**) at 45℃ or water saturated n-hexane / 2-propanol (50:1, v/v; for **10,11**) at 25℃. In both cases, the mobile phases were eluted at 1.0 ml/min. All diastereomers tested were separated with Rs values of more than 0.87 even though the diastereomers of **11** have seven-bonds remote chiral centers. Here, the use of water saturated solvent as a mobile phase was very effective for the chiral separation.

We have thus developed a chiral fluorescent derivatization reagent that made it possible to separate the enatiomers of carboxylic acids having asymmetric centers at remote positions from their carboxyl groups.

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3. Akasaka,K., Ohrui,H., Meguro,H. and Yashumoto,T., *J.Chromatogr.A*, **1996**, 729, 381- 386.
4. Akasaka,K., Ohrui,H., Meguro,H. and Yashumoto,T., *Anal.Sci.*, **1996**, 12, 557- 560.
5. (S)-**9** : mp. 293℃, Hi-MASS; m/z=437.0545 (Calcd. for $C_{20}H_{14}O_5NSF_3$=437.0544)
6. (S)-(+)-2-amino-1-propanol was purchased from Fluka-Biochemika Analytika.

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