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Note

Resolution of enantiomers of 19-hydroxyeicosatetraenoate and 18-hydroxyeicosatetraenoate by chiral phase high-performance liquid chromatography of naphthoyl ester derivatives

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Arachidonic acid is metabolized to *cis–trans*-conjugated hydroxyeicosatetraenoic acids (HETEs) by mammalian and plant lipoxygenases [1]. Kühn et al. [2] have demonstrated that methyl esters of a number of *cis–trans*-conjugated HETEs can be partially resolved by chiral phase high-performance liquid chromatography (HPLC) using (*R*)-(−)-N-3,5-dinitrobenzoyl-α-phenylglycine (DNBPG) ionically bound to the stationary phase (Pirkle type 1-A). The method allows steric analysis of a series of lipoxygenase-derived HETEs and it has already been widely used [3–5]. The separation is presumed to be due to π–π donor–acceptor interactions between the solute enantiomers and the 'electron-deficient' dinitrobenzoyl residues of the stationary phase, as well as hydrogen bonding and other steric effects [6]. Hawkins et al. [7] recently showed that an improved separation of the isomers of 5-HETE, 8-HETE, 11-HETE, 12-HETE and 15-HETE was achieved on the DNBPG column by using 'electron-rich' naphthoyl ester derivatives of HETE methyl esters. Other chiral phases may also be useful for separation of HETEs, and the steric isomers of methyl 12-HETE were recently reported to be resolved on a cellulose trisphenylcarbamate stationary phase [8].

Arachidonic acid is metabolized by ω3-, ω2- and ω1-hydroxylation to 18-HETE, 19-HETE and 20-HETE, respectively, by mammalian and fungal cytochrome P-450 [9–12]. The fungus *Ophiobolus graminis* thus converts exogenous arachidonic acid into 18(*R*)-HETE and 19(*R*)-HETE in good yields

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[9,10]. 18(*R*)-HETE is also formed by a prominent ω3-hydroxylase of monkey seminal vesicles [10], and 19-HETE and 20-HETE are synthesized in the liver and renal cortex of rodents [11,12]. 18(*R*)-HETE and 19(*R*)-HETE are biologically active on organ preparations in vitro (Dahlén and Oliw, unpublished results). Furthermore, 19(*S*)-HETE is reported to be a potent stimulator of renal Na^+–K^+-ATPase in vitro [13].

In view of the widespread use of chiral phase HPLC for steric analysis of HETEs with a conjugated *cis–trans* functionality, it seemed of interest to determine whether these methods could be extended to ω2- and ω3-hydroxy metabolites of arachidonic acid, which lack conjugated double bonds. A large number of chiral phase HPLC columns are commercially available and most are expensive [6]. Two chiral HPLC columns with proven capacity to resolve HETEs with conjugated double bonds were evaluated in the present report, i.e. DNBPG and cellulose trisphenylcarbamate.

EXPERIMENTAL

Chromatography

The HPLC apparatus consisted of a pump (Milton Roy CM 4000), an injector (Rheodyne 7125), a variable-wavelength UV detector (Kratos Spectroflow 757) and an integrator (Hitachi 2500). The chiral phase HPLC columns (250 mm × 4.6 mm I.D.) were (i) DNBPG ionically linked to 5 μm modified Spherisorb (Pirkle type 1-A, Regis, Morton Grove, IL, U.S.A.) and (ii) cellulose trisphenylcarbamate bound to silica gel (Chiralcel OC, Diacel, Tokyo, Japan). Naphthoyl ester derivatives were monitored by UV absorbance at 221 nm or, occasionally, at 300 nm as described previously [7]. The columns were eluted isocratically (1.0 ml/min) at room temperature with a mobile phase containing 0.25 or 0.5% 2-propanol in hexane, respectively.

Reagents and chemicals

Arachidonic acid was purchased from Nu-Check-Prep (Elysian, MN, U.S.A.) and from Sigma (St. Louis, MO, U.S.A.). 19(*R*)-HETE and 18(*R*)-HETE were obtained as described previously by fungal biosynthesis [9] using *O. graminis* (*Gaeumannomyces graminis*), which was purchased from Centraalbureau voor Schimmelcultures (Baarn, The Netherlands). 19-HETE and 18-HETE were purified by reversed- and normal-phase HPLC as described previously and characterized by gas chromatography–mass spectrometry (GC–MS) [10]. Steric analysis of these compounds was performed by capillary GC–MS after hydrogenation, methylation and conversion into 2(*S*)-phenylpropionic acid derivatives according to the method of Hammerström and Hamberg [14]. The results showed that they contained at least 90% of the *R*-isomer [10]. Racemic 19-HETE methyl ester and racemic 18-HETE methyl ester were obtained from the *R*-isomers by oxidation of the hydroxyl group to a ketone

with CrO_3 in pyridine [15], followed by reduction of the ketone with $NaBH_4$ and GC–MS analysis of the products [10]. 18(*S*)-HETE methyl ester and 19(*S*)-HETE methyl ester were synthesized from 18(*R*)-HETE methyl ester (4 mg) and 19(*R*)-HETE methyl ester (4 mg) essentially as described previously [13]. The benzoyl ester derivatives of 19-HETE and 18-HETE methyl esters were purified by preparative thin-layer chromatography (TLC) (silica gel, 0.25 mm thickness, Merck) using 30% (v/v) ethyl acetate in hexane [13] and hydrolysed with 0.5 *M* potassium hydroxide in methanol–water (10:1, v/v) under argon (1 h, 70°C). After extractive isolation and methylation, the products were purified by normal-phase HPLC [10], and the procedure yielded ca. 0.5 mg of the methyl esters of 18(*S*)-HETE and 19(*S*)-HETE.

1-Naphthoyl chloride (98%) was from Aldrich-Chemie (Steinheim, F.R.G.) and naphthoyl ester derivatives were prepared essentially as described previously [7]. 19-HETE methyl ester (50–100 μg) or 18-HETE methyl ester (50–100 μg) were dissolved in dry pyridine (20 μl) and 40 μl of 1 *M* naphthoyl chloride in dry toluene were added. After at least 30 min, the solvents were evaporated to dryness and the residue was dissolved in chloroform and applied to a cartridge of silica (Sep-Pak, Waters Assoc., Milford, MA, U.S.A.). The naphthoyl ester derivatives were eluted with 4–5 ml of chloroform, which was evaporated to dryness. The residue was dissolved in hexane with 0.5% 2-propanol and centrifuged briefly (11 000 *g*). Subsequently 10–20 μl of the supernatant were analysed and purified by chiral phase HPLC (DNBPG). The purified 19-HETE and 18-HETE derivatives were then further analysed by HPLC on cellulose trisphenylcarbamate. The naphthoyl ester derivatives of 18-HETE and 19-HETE showed UV absorbance maxima at 221 nm and 300 nm [7].

RESULTS AND DISCUSSION

Naphthylated 19(*R,S*)-HETE methyl ester and naphthylated 18(*R,S*)-HETE methyl ester were partially resolved on the DNBPG stationary phase, as illustrated in Fig. 1. In both cases the *R*-isomer eluted before the *S*-isomer (Fig. 1). This elution order is identical with the elution of the *R*- and *S*-isomers of naphthylated 5-, 8-, 11-, 12- and 15-HETE methyl esters, but the reverse of the order of the isomers of the corresponding methyl ester derivatives [7]. The elution volumes of naphthoyl ester derivatives of 18(*R*)-HETE methyl ester, 18(*S*)-HETE methyl ester, 19(*R*)-HETE methyl ester and 19(*S*)-HETE methyl ester were ca. 23.0, 23.6, 27.4, and 28.4 ml, respectively, using 0.25% 2-propanol in hexane as eluent. The resolution factors [16] of the 19(*R,S*)-HETE and 18(*R,S*)-HETE derivatives were 0.9 and 0.7, respectively.

Naphthylated 19(*R,S*)-HETE methyl ester was partially resolved by chromatography on the stationary cellulose trisphenylcarbamate column, as illustrated in Fig. 2. The *S*-isomer eluted before the *R*-isomer. This is in contrast to the elution order of underivatized 12(*R,S*)-HETE methyl ester, in which

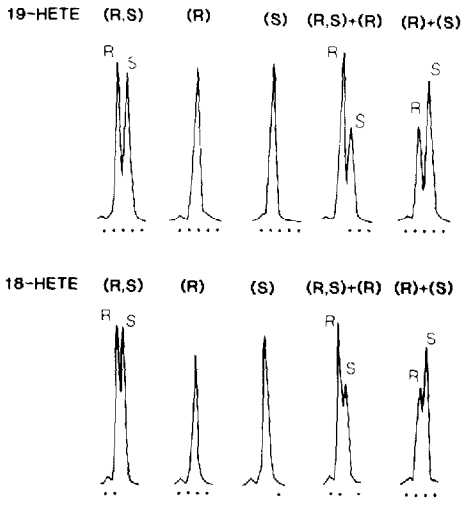

Fig. 1. Partial chromatograms of naphthoyl ester derivatives of enantiomers of 19-HETE methyl ester (top) and 18-HETE methyl ester (bottom), which were separated by chiral phase HPLC (DNBPG stationary phase) The compounds were detected by their UV absorbance at 221 nm and the time scales on the abscissa are in minutes (the interval between two dots is 1 min) The column was eluted with 0.25% (v/v) 2-propanol in hexane (1.0 ml/min). The retention time of the 19(*R*)-HETE derivative was 27.4 min and that of the 18(*R*)-HETE derivative was 23 0 min.

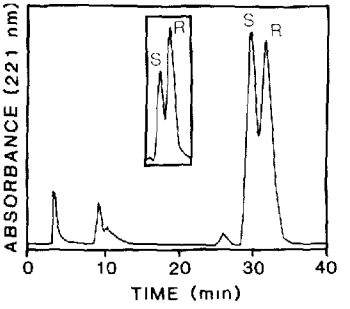

Fig 2 Analysis of the naphthoyl ester derivative of 19(*R,S*)-HETE methyl ester by chiral phase HPLC (cellulose trisphenylcarbamate, Chiralcel OC). The inset shows a partial chromatogram from analysis by chiral phase HPLC of a mixture of the same derivatives of 19(*R,S*)-HETE and 19(*R*)-HETE. The column was eluted with 0.5% (v/v) 2-propanol in hexane (1.0 ml/min).

case the *R*-isomer elutes before the *S*-isomer [8]. The resolution factor of the 19-HETE derivative was 0.6. Naphthylated 18(*R,S*)-HETE methyl ester was not resolved under the present conditions (0.25% 2-propanol in hexane, elution volume 23 ml).

These results demonstrate that the enantiomers of 19(*R,S*)-HETE can be resolved as naphthoyl ester derivative by chiral phase HPLC using either DNBPG or cellulose trisphenylcarbamate as stationary phase. In contrast, the

resolution of the enantiomers of the same derivative of 18(*R,S*)-HETE methyl ester was less satisfactory than the resolution of 19(*R,S*)-HETE on the DNBPG column. The isomers of 18(*R,S*)-HETE were not resolved on the cellulose trisphenylcarbamate stationary phase. So far, 18-HETE has only been demonstrated as a mammalian metabolite of arachidonic acid in seminal vesicles of the cynomolgus monkey (*Macaca fascicularis*) and the enzymic product has been found to be the 18(*R*)-HETE isomer [10]. 19-HETE is formed from arachidonic acid by hepatic and renal cortical cytochrome P-450 and no steric analysis of this metabolite has been reported. This could be of interest in view of the stimulatory effects of 19(*S*)-HETE on renal Na^+–K^+-ATPase and the lack of effect of 19(*R*)-HETE on this enzyme [13]. The present method of resolving the enantiomers of 19(*R,S*)-HETE could be useful for steric analysis of 19-HETE formed by the renal cortex and by other tissues.

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REFERENCES

1 P Needleman, J Turk, J. Jakschik, A.R. Morrison and J B. Lefkowith, Annu. Rev. Biochem., 55 (1986) 69.
2 H Kühn, R. Wiesner, V Z. Lankin, A Nekrasov, L. Adler and T. Schewe, Anal. Biochem., 160 (1987) 24.
3 D.J Hawkins and A.R. Brash, J Biol. Chem , 262 (1987) 7629
4 J. Turk, T Stump, B A. Wolf, R A Easom and M.L. McDaniel, Anal. Biochem., 174 (1988) 580.
5 E.H Oliw and H Sprecher, Biochim. Biophys Acta, 1002 (1989) 283.
6 R. Dappen, H. Arm and V R Meyer, J. Chromatogr., 373 (1986) 1.
7 D J Hawkins, H Kühn, E.H Petty and A.R. Brash, Anal. Biochem., 173 (1988) 456.
8 S. Kitamura, T. Shimizu, I. Miki, T. Izumi, T. Kasama, A. Sato, H Sano and Y. Seyama, Eur. J Biochem., 176 (1988) 725.
9 C J. Sih, G. Ambrus, P Foss and C J. Lai, J. Am Chem. Soc., 91 (1969) 3685.
10 E H. Oliw, J. Biol. Chem , 264 (1989) 17 845
11 E.H Oliw, J.A Lawson, A R. Brash and J.A. Oates, J. Biol Chem., 256 (1981) 9924.
12 E H Oliw and J.A. Oates, Biochim. Biophys. Acta, 666 (1981) 327
13 B Escalante, J.R Falck, P. Yadagiri, L. Sun and M. Laniado-Schwartzman, Biochem. Biophys. Res., Commun , 152 (1988) 1269
14 S Hammarstrom and M. Hamberg, Anal Biochem., 52 (1973) 169
15 R Ratcliffe and R. Rodehorst, J. Org. Chem., 35 (1970) 4000.
16 C.F. Simpson, Practical High-Performance Liquid Chromatography, Heyden, London, 1978, p. 10.