ELSEVIER

Journal of Chromatography A, 966 (2002) 221–225

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

# Chiral liquid chromatography separation and chiroptical properties of the enantiomers of dimethyl α-hydroxyfarnesylphosphonate, a precursor of a farnesyl protein transferase inhibitor

Salvatore Caccamese[a,*], Giovanna Scivoli[a], Yanming Du[b], David F. Wiemer[b]

[a]*Dipartimento di Scienze Chimiche, Università di Catania, viale A. Doria 6, 95125 Catania, Italy*

[b]*Department of Chemistry, University of Iowa, Iowa City, IA 52245, USA*

Received 12 March 2002; received in revised form 17 May 2002; accepted 24 May 2002

**Abstract**

The HPLC enantiomeric separation of racemic and non-racemic samples of dimethyl α-hydroxyfarnesylphosphonate (**1**) was accomplished using Chiralcel OD as chiral stationary phase. Single enantiomers were isolated by semipreparative HPLC and their CD spectra and optical rotations were measured. The method ascertains enantiomeric excess of **1**, obtained by oxidation of dimethylfarnesylphosphonate with enantiopure oxaziridines, avoiding converting the enantiomers to diastereomers by the use of a chiral auxiliary. Stability of the solutions of **1** is strongly dependent on the nature of the solvent.


*Keywords:* Enantiomer separation; Circular dichroism spectra; Chiral stationary phase, LC; Dimethyl α-hydroxyfarnesylphosphonate; Hydroxyphosphonates

## 1. Introduction

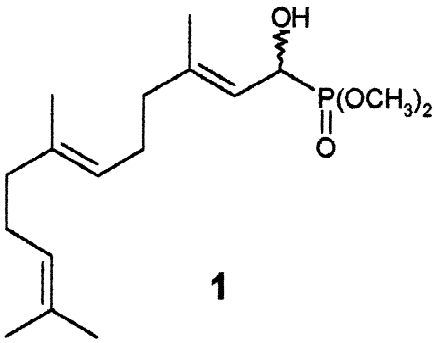
Various α-hydroxyphosphonates have shown activity as inhibitors of a variety of enzymes including renin [1], enolpyruvylshikimate-3-phosphate (EPSP) synthase [2], farnesyl protein transferase (FPTase) [3], protein tyrosine kinase [4], and CD-45 tyrosine phosphatase [5]. α-Hydroxyfarnesylphosphonic acid, in its racemic form, has been shown to inhibit FPTase with an $IC_{50}$ of 30 nm [3].

Due to the importance of the absolute configuration in the α-position of substituted phosphonic acids to biological activity [6], preparation of individual enantiomers of α-hydroxyfarnesyl phosphonic acid by hydrolysis of its dimethyl ester **1** was studied [7]. However, hydrolysis was accompanied by extensive racemization. Also the description of the enantiomeric purity of **1**, obtained as reaction product in the oxidation of dimethylfarnesylphosphonate with chiral camphorsulfonyloxaziridine, was done via further reaction of the ester **1** with (*S*)-*O*-methylmandelic acid and integration of the resonances of the obtained diastereomers in the $^{31}P$ NMR spectrum [7].

*Corresponding author. Tel.: +39-095-738-5029; fax: +39-095-580138.

*E-mail address:* scaccamese@dipchi.unict.it (S. Caccamese).

0021-9673/02/$ – see front matter 
PII: S0021-9673(02)00736-7




1

Due to the experience of some of us in the enantiomeric separation of several α-hydroxybenzylphosphonate diethyl-esters [8] and fluorinated *N*-arylamino-1-arylmethyl-phosphonates [9] using chiral HPLC, we decided to attempt the separation and isolation of pure enantiomers of dimethyl α-hydroxyfarnesylphosphonate **1**. The lack of aromatic moieties or conjugated system in compound **1** was a point of difficulty with respect to the previously studied compounds where variously substituted aromatic moieties helped in the chiral recognition process by the chiral stationary phase (CSP).

In this paper, we report the direct separation of the enantiomers of phosphonate **1** and the chiroptical properties (CD spectra and optical rotation) of the isolated enantiomers.

## 2. Experimental

### *2.1. Instrumentation*

The HPLC system consisted of a Varian 5060 liquid chromatograph with Valco 10- or 50-μl sample loops, a Jasco Uvidec 100-III UV spectrophotometric detector operating at 225 nm and a Varian DataJet integrator or Houston Omniscribe recorder for fraction collecting. Circular dichroism spectra were recorded on a Jasco 810 spectropolarimeter. Optical rotations were measured with a Jasco DIP-730 digital polarimeter using a 10-cm microcell. The polysaccharide columns (250×4.6 mm) were Chiralcel OD (cellulose tris-3,5-dimethylcarbamate) and Chiralpak AD (amylose tris-3,5-dimethylphenylcarbamate) both coated on 10 μm silica gel from Daicel (Tokyo). The Pirkle type columns (250×4.6 mm) were (3*S*,4*R*)-Whelk O-1 [4-(3,5 dinitrobenzamido)-tetrahydrophenanthrene] covalently bonded to 5 μm 3-propyl silica and (*R*)-α-Burke 1 [dimethyl *N*-3,5-dinitrobenzoyl-α-amino-2,2-dimethyl-4-pentylphosphonate] covalently bonded to 5 μm mercaptopropyl silica, both from Regis Chemical (Morton Grove, IL, USA). The column dead time ($t_0$) was measured by injection of tri-*tert*.-butylbenzene as a non-retained sample [10]. Other HPLC chromatographic parameters were those typically employed [11]. All separations were carried out at room temperature.

### *2.2. Chemicals*

The synthesis and full characterization of dimethyl α-hydroxyfarnesylphosphonates have been reported elsewhere, as non-racemic [7] and racemic [3] compounds, respectively.

## 3. Results and discussion

The chromatographic results are presented in Table 1 and typical chromatograms are shown in Fig. 1. The most efficient CSP by far is the Chiralcel OD, which gave separation factors $\alpha$ in the range 1.37–1.25 using 2-propanol as polar modifier of the mobile phase. Indeed, Chiralpak AD, using the same mobile phase composition (*n*-hexane/2-propanol 95:5) and flow-rate was totally ineffective. It is well known that the difference in the size of the helical cavity of the cellulose and amylose-derived phases (Chiralcel OD and Chiralpak AD, respectively) [12,13] plays a significant role in the chiral recognition of many classes of compounds and this can explain the observed behavior of compound **1**. The two Pirkle CSP (Whelk O-1 and α-Burke) used by us were much less effective, giving poor separation factors of 1.04 and 1.01, respectively, and, more important, the resolution factor $R_s$ was totally unsatisfactory. The Whelk O-1 CSP had instead been very successful in the enantiomeric separation of α-hydroxybenzylphosphonate esters where an interaction between the π-acid dinitrobenzamide group of the CSP and the π-basic aromatic group of the analyte was present [8].

Other features that can be extracted from the results in Table 1 and Fig. 1 are that: (i) a decrease

Table 1
HPLC behavior of racemic dimethyl α-hydroxyfarnesylphosphonate on various chiral stationary phases

| CSP | $A$ (%)[a] | $FR$[b] | $t_1$ (min) | $t_2$ (min) | $k_1$[c] | $\alpha$[d] | $R_s$ |
|---|---|---|---|---|---|---|---|
| Chiralcel OD | 10 | 0.7 | 6.0 | | 0.30 | NS[e] | |
| OD | 5 | 0.7 | 10.8 | 12.3 | 1.33 | 1.25 | 1.0 |
| OD | 3 | 0.7 | 14.5 | 17.5 | 2.11 | 1.31 | 1.1 |
| OD | 5 | 1.0 | 7.3 | 8.3 | 1.11 | 1.25 | 0.8 |
| OD | 3[f] | 1.0 | 11.5 | 14.5 | 2.28 | 1.37 | 1.4 |
| OD | 5[g] | 1.0 | 6.2[h] | | 0.76 | NS[e] | |
| OD | 2[g] | 1.0 | 10.8 | 11.5 | 2.08 | 1.10 | <0.4 |
| Chiralpak AD | 5 | 0.7 | 9.4 | | 1.03 | NS[e] | |
| Whelk O-1 | 5 | 0.7[i] | 20.8 | 21.6 | 3.65 | 1.04 | <0.4 |
| α-Burke | 2 | 1.0[j] | 23.2 | 23.6 | 6.81 | 1.01 | <0.4 |

[a] Percentage of 2-propanol in *n*-hexane, unless otherwise specified, with UV detector at 225 nm.
[b] Flow rate (ml/min); $FR=0.7$, $t_0=4.63$ min; $FR=1$, $t_0=3.50$ for OD and AD columns.
[c] Retention factor of the first-eluted enantiomer.
[d] Separation factor.
[e] Not separated.
[f] Experimental conditions used for semipreparative isolation.
[g] Percentage of ethanol in *n*-hexane.
[h] Shoulder in the descending edge of the peak.
[i] $t_0=4.47$ min.
[j] $t_0=2.97$ min.

in the polarity of the mobile phase (*n*-hexane/2-propanol or *n*-hexane/ethanol) has a beneficial effect on the separation factor $\alpha$ while the resolution factor $R_s$ is almost unaffected; and (ii) the use of ethanol as polar modifier of the mobile phase reduces the retention of both enantiomers but strongly reduces the separation factor.

The chiroptical behavior of the enantiomers of

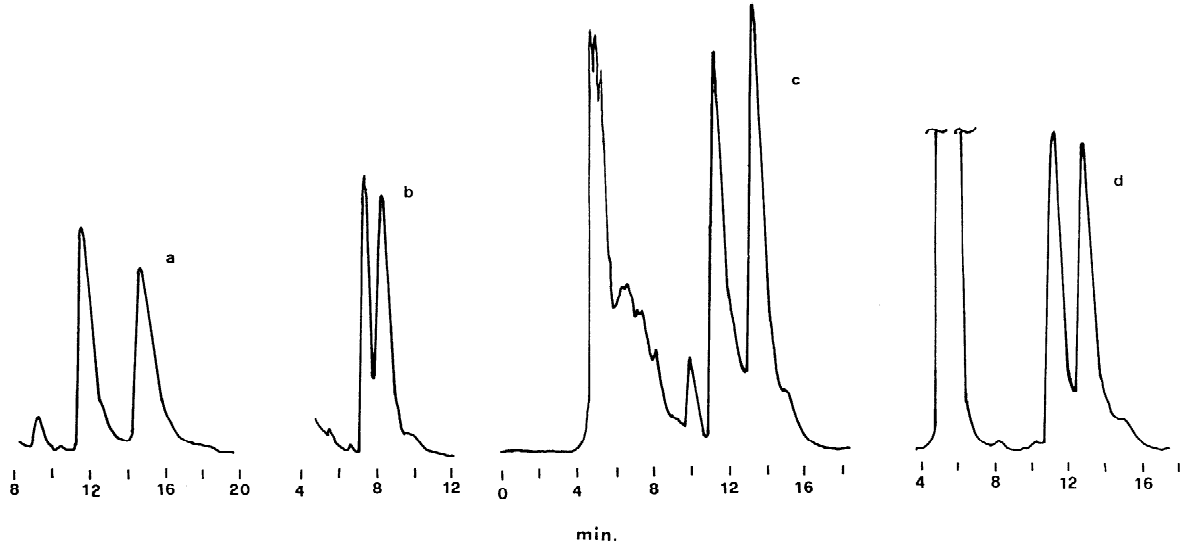



Fig. 1. Typical HPLC separation of the enantiomers of racemic **1**. Conditions: stationary phase Chiralcel OD; mobile phase *n*-hexane/2-propanol (a) 97:3, (b, c, d) 95:5, at 1 ml/min (a, b) and 0.7 ml/min (c, d). Solutions ''a'' and ''b'', freshly prepared in *n*-hexane/2-propanol 1:1, solution ''c'' same as ''a'' after 3 h, solution ''d'' in $CCl_4$ after 3 h. Truncated peak in ''d'' is $CCl_4$.

dimethyl α-hydroxyfarnesylphosphonate was obtained after their isolation. In fact, using the experimental parameters reported in Table 1 for semi-preparative chromatography, which are a compromise between acceptable elution times and resolution factors, repeated 50-μl injections (0.2–0.3 mg) of racemic **1** and separate collection of the eluates corresponding to the two chromatographic peaks afforded the isolation of the individual enantiomers. The CD spectra of both eluates were measured and they were mirror images of each other, as shown in Fig. 2, indicating their enantiomeric nature. The spectra were recorded in *n*-hexane and the absorbance was found to be stable after 3 h after the first acquisition of the spectrum. Analytical HPLC reruns of the eluates indicated an enantiomeric excess (ee) of 92% for the first peak and 77.8% for the second peak. The Cotton effect is present at low wavelengths (peaks centered at 200–205 nm) and has a low intensity, as expected for a molecule which does not possess a conjugated chromophore.

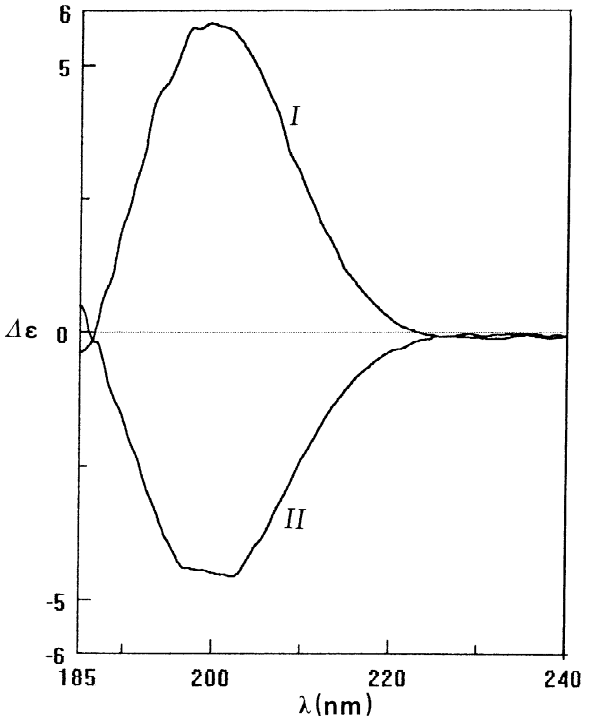



Fig. 2. CD spectra (*n*-hexane, 22 °C) of the enantiomers of compound **1**, obtained from the first (I) and second (II) HPLC eluted peaks.

A specific rotation $[\alpha]_D^{22}$ +37.1 (*c* 1.45, $CCl_4$) was measured for the first-eluted sample of compound **1**, while the second one afforded an experimental $[\alpha]_D^{22}$ −29.6 (*c* 1.79, $CCl_4$), in agreement with its smaller ee as measured by chiral HPLC.

Using the values of ee and [α] obtained for the eluted peaks, values of [α]=40.3 and −38.0 for the first and second enantiomer, respectively, were calculated for ee 100%. The slight discrepancy in these values probably originated from inaccuracy in the determination of the areas of the HPLC peaks due to the presence of small spurious peaks in the baseline.

The values of [α] and ee observed by us for the single enantiomers are much higher than those obtained for earlier enantioenriched samples of (+)- and (−)-**1** [7]. Moreover, Wiemer et al. obtained ee values by indirect analysis of the $^{31}$P NMR spectra of the diastereomeric (*S*)-*O*-methylmandelates of **1**.

Because the absolute stereochemistry of the α-carbon in the diastereomeric (*RS*)- and (*SS*)-*O*-methylmandelate esters was assigned on the basis of shifts observed in the $^{1}$H and $^{31}$P NMR spectra and it was related to the sign of the specific optical rotation [7], by comparison with the [α] obtained by us it was possible to assign the stereochemistry of the first eluted enantiomer of compound **1** as possessing the (*R*) configuration.

We also examined a non-racemic sample of compound **1**, obtained by oxidation of dimethylfarnesylphosphonate with a chiral auxiliary (camphorsulfonyloxaziridine), in the conditions reported in Table 1, and we obtained an ee of (*S*)−**1** of 47%, corresponding to a percentage of 73.5. The value reported for the ee of this sample of (*S*), from calculations based on the integration of the $^{31}$P NMR spectrum of the (*S*)-*O*-methylmandelate was instead 55%, with an [α] observed of −20.2 [7].

The samples of racemic and non-racemic compound **1** are oils and they solidify easily at subambient temperature. In this form they can be stored for months at −18 °C without appearance of decomposition products in the chiral HPLC. However, the stability of the solutions is strongly dependent on the

nature of the solvent. In fact, as shown in Fig. 1c, a solution in *n*-hexane/2-propanol 1:1 shows shoulders and additional decomposition peaks in the chiral HPLC trace at lower elution times with respect to the enantiomers of **1** after 3 h from preparation. It is likely that these peaks arise from a transesterification process caused by the presence of 2-propanol in the mobile phase. Solutions prepared in $CCl_4$ are instead much more stable at the same interval time, as shown in Fig. 1d. This may be due to a different polarity index of the solvents used. In any event, the choice of the solvent is important for chiroptical measurements of this kind of compounds.

In summary, our results can be used to ascertain accurately the enantiomeric excess of the α-hydroxy-farnesylphosphonates obtained by asymmetric synthesis avoiding the need to convert the enantiomers to diastereomers by the use of a chiral auxiliary. This will speed the selection of the experimental conditions (chiral oxaziridine, base, temperature) which give the highest ee. The racemization of an enantiopure dimethyl-ester occurring during hydrolysis can also be studied and, eventually, avoided. In addition, the chiroptical properties of nearly pure enantiomers of compound **1** can be used as comparison values.

## References

[1] D.V. Patel, K. Rielly-Gauvin, D.E. Ryono, Tetrahedron Lett. 31 (1990) 5587.
[2] J.A. Sikorski, M.J. Miller, D.S. Braccolino, D.G. Cleary, S.D. Corey, J.L. Font, K.J. Gruys, C.Y. Han, K.C. Lin, P. Pansegrau, J.E. Ream, D. Schnur, A. Shah, M.C. Walker, Phosphorus Sulfur Silicon 76 (1993) 375.
[3] D.L. Pompliano, E. Rands, M.D. Schaber, S.D. Mosser, N.J. Anthony, J.B. Gibbs, Biochemistry 31 (1992) 3800.
[4] T.R. Burke, Z.H. Li, J.B. Bolen, V.E. Marquez, J. Med. Chem. 34 (1991) 1577.
[5] R.F. Frechette, C. Ackerman, S. Beers, R. Look, J. Moore, Biorg. Med. Chem. Lett. 7 (1997) 2169.
[6] P. Kafarski, B. Lejczak, Phosphorus Sulphur Silicon 63 (1991) 193.
[7] D.M. Cermak, Y. Du, D.F. Wiemer, J. Org. Chem. 64 (1999) 388.
[8] S. Caccamese, S. Failla, P. Finocchiaro, G. Principato, Chirality 10 (1998) 100.
[9] S. Caccamese, G. Principato, U. Gruss, G. Hägele, S. Failla, Tetrahedron Asymmetry 5 (1994) 141.
[10] W.H. Pirkle, C.J. Welch, J. Liq. Chromatogr. 14 (1991) 1.
[11] S. Allenmark, Chromatographic Enantioseparation: Methods and Applications, 2nd ed, Ellis Horwood, Chichester, 1991, p. 82.
[12] Y. Okamoto, E. Yashima, Angew. Chem., Int. Ed. 37 (1998) 1020.
[13] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.