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Note

Determination of enantiomeric purity of *Z*-oxylysine by capillary gas chromatography

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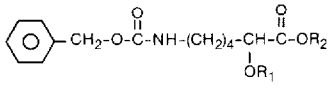
The L enantiomer of *Z*-oxylysine (compound I, Table I) is a key intermediate in the synthesis of a new angiotensin converting enzyme (ACE) inhibitor used in the treatment of hypertension[1,2]. An optical purity method was required to determine the optical purity of the desired L enantiomer. The enantiomeric separation by gas chromatography (GC) employs chiral reagent–achiral GC column[3] or achiral reagent–chiral GC column combination[4–7]. While the latter approach is preferred, the direct separation of the enantiomers of *Z*-oxylysine with commercially available chiral column was not feasible. A GC method using a chiral reagent for the diastereomer formation and a capillary achiral column for chromatography is described. As little as 0.2% of the D enantiomer in a sample of L-*Z*-oxylysine can be determined.

TABLE I

CHEMICAL STRUCTURES OF *Z*-OXYLYSINE AND ITS DERIVATIVES



Compound	R_1	R_2
I (*Z*-oxylysine)	H	H
II	H	$-\underset{\displaystyle CH_3}{\overset{\displaystyle H}{\underset{\displaystyle |}{\overset{\displaystyle |}{CH}}}}-(CH_2)_5-CH_3$
III	H	$-\underset{\displaystyle CH_3}{\underset{\displaystyle |}{CH}}-CH_2-CH_3$ (butyl)
IV	$-\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}-CF_2CF_3$	butyl
V	$-Si(CH_3)_3$	butyl
VI	$-Si(CH_3)_2\ C(CH_3)_3$	butyl
VII	$-\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}-NH-CH(CH_3)_2$	butyl

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EXPERIMENTAL

Gas chromatography

A Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector and an autosampler was used. Chromatography was performed on a DB-17+, 15 m × 0.32 mm I.D. with a 0.5 μm stationary phase film thickness (J&W). The inlet pressure of the helium carrier gas was 90 KPa (13 p.s.i.g.) and the flow-rate of the helium make-up gas for the flame ionization detector was 25 ml/min. Injections were made in the split mode at a split flow-rate of 50 ml/min, using an empty glass insert (Hewlett-Packard). The oven temperature was held isothermally at 240°C. The injector and detector temperatures were maintained at 280°C.

Reagents and chemicals

The L and D enantiomers of *Z*-oxylysine were characterized reference materials obtained from the Department of Chemical Process Technology (E. R. Squibb & Sons). Thionyl chloride, *l*-2-octanol and *d*-2-octanol were obtained from Aldrich. A thionyl chloride solution was prepared by dissolving 2.5 ml of thionyl chloride and 2.5 μl of dimethylformamide in 25 ml of *n*-hexane. The solution of *l*-2-octanol was prepared by dissolving 2.5 ml of *l*-2-octanol in 25 ml of methylene chloride.

Sample preparation

To approximately 6 mg of L-*Z*-oxylysine sample, weighed directly into an autosampler vial, 1.0 ml of thionyl chloride solution was added. The vial contents, sealed with a PTFE-lined cap, were vortexed and kept at room temperature for 30 min. The vial was then uncapped and the reagent was removed by evaporation at 50°C under a stream of nitrogen. To the dried residue, 0.3 ml of *l*-2-octanol solution was added to the vial. After sealing and vortexing, the solution was heated at 60°C for 30 min. The cooled vial was then uncapped and the reagent was removed by evaporation at 50°C, under nitrogen. The samples, reconstituted with 0.5 ml of methylene chloride, were placed into the autosampler after recapping the vials. A 1.0-μl aliquot was then injected.

Quantitation

The percentage of the L enantiomer in the L-*Z*-oxylysine sample was calculated using the formula:

$$L = \frac{P(A_1 + A_2) - A_1}{(A_1 + A_2)(2P - 1)} \cdot 100$$

where L = percentage of the L enantiomer of *Z*-oxylysine; P = percentage of the *l* enantiomer of 2-octanol divided by 100; A_1 = area of peak 1 (Fig. 1 and Appendix); A_2 = area of peak 2 (Fig. 1 and Appendix)

RESULTS AND DISCUSSION

The method utilizes a chiral reagent for derivatization of *Z*-oxylysine (compound I, Table I) and achiral fused-silica capillary column for chromatographic sep-

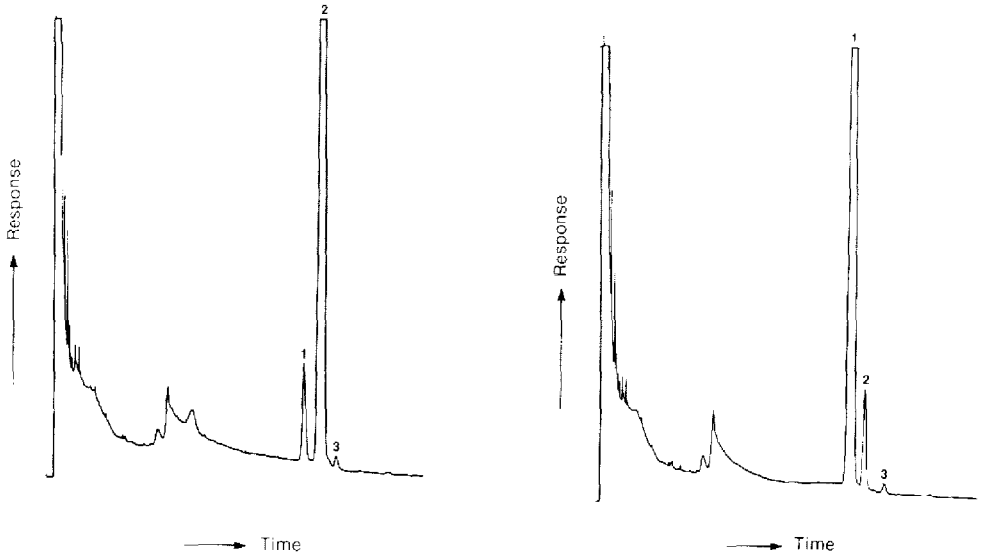

Fig. 1. Chromatogram of L-*Z*-oxylysine sample spiked with 3.0% of D-*Z*-oxylysine, after esterification with *l*-2-octanol. Peak 1 = D-*Z*-oxylysine (16.3 min); peak 2 = L-*Z*-oxylysine (17.5 min); peak 3 = unknown.

Fig. 2. Chromatogram of L-*Z*-oxylysine sample spiked with 3.0% D-*Z*-oxylysine, after esterification with *d*-2-octanol. Peak 1 = L-*Z*-oxylysine (16.3 min); peak 2 = D-*Z*-oxylysine (17.5 min); peak 3 = unknown.

aration. Compound I is first converted into the acid chloride with thionyl chloride which is then reacted with the chiral *l*-2-octanol to form diastereomeric esters (compound II, Table I). GC using a DB-17+ achiral capillary column is used to resolve the octyl diastereomeric esters (Figs. 1 and 3).

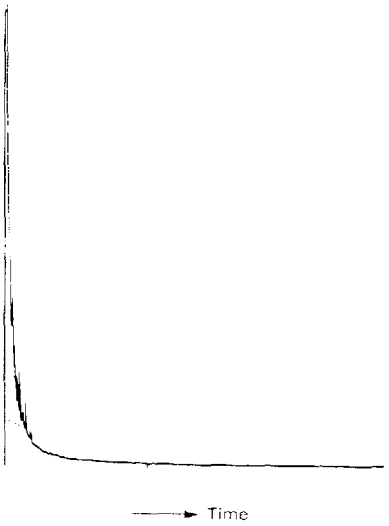

Fig. 3. Chromatogram of a reagent blank corresponding to Fig. 1.

TABLE II

RECOVERY OF D-Z-OXYLYSINE ADDED TO L-Z-OXYLYSINE

The values shown in parentheses are obtained after correction for concentration from the L enantiomer sample.

Added (%)	*Recovered (%)*
0	0.53 (0)
0.50	1.03 (0.50)
1.49	2.08 (1.55)
4.98	5.59 (5.06)

By reacting L-*Z*-oxylysine with *l*-2-octanol and *d*-2-octanol, separately, it was established that the L*l* and L*d* diastereomers are chromatographically separable and that L*l* elutes after L*d* (Figs. 1 and 2). By reacting D-*Z*-oxylysine with *l*-2-octanol and *d*-2-octanol, it was also shown that D*d* elutes after D*l*. As expected for a non-chiral column, L*l* co-eluted with D*d* and L*d* co-eluted with D*l*. For the determination of a trace amount of the D enantiomer in L-*Z*-oxylysine sample, *l*-2-octanol was used as the reagent, since the minor peak will elute before the major peak to yield a more accurate measurement.

The two reactions used for sample preparations, acid chloride formation and octyl ester formation, were studied by varying reaction times. No difference was seen in % D of L-*Z*-oxylysine when each reaction time was increased up to four hours. Consequently, no racemization occurs.

The accuracy of the method was established by analyzing L-*Z*-oxylysine samples spiked with varying amounts of D-*Z*-oxylysine. As shown in Table II, added D-*Z*-oxylysine was quantitatively recovered. Excellent precision was obtained for replicate sample preparations of a batch of L-*Z*-oxylysine (Table III). The limit of quantitation was estimated to be 0.2% of the D enantiomer.

Various derivatization reactions were investigated. Esterification with *d*-2-butanol following acid chloride formation with thionyl chloride gave compound III (Table I), but there was no separation between the butyl ester diastereomers on the achiral column. Compound IV, formed by acylation of III with pentafluoropropionic

TABLE III

REPRODUCIBILITY OF REPLICATE SAMPLE PREPARATIONS OF A BATCH OF L-*Z*-OXYLYSINE

R.S.D. = Relative standard deviation.

Replicate No.	*% D*
1	0.63
2	0.68
3	0.67
4	0.63
5	0.69
Average	0.66
R.S.D.	4.5%

anhydride (PFPA), eluted earlier than III, but the diastereomers could not be resolved. Silylation of III with trimethylsilylimidazole in pyridine (Tri-Sil Z) or N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) to form V and VI, respectively, did not achieve the desired separation. Carbamate formation reaction[4,7] of III with 0.5 ml of isopropyl isocyanate was nearly complete with triethylamine, 10 μl, used as a catalyst, at 60°C, 30 min. The diastereomers (VII) were not resolved, however.

There was no resolution between the diastereomers of II when the alcoholic group was acylated or silylated, with PFPA, Tri-Sil Z or MTBSTFA. The carbamate product formed when II was reacted with isopropyl isocyanate and triethylamine eluted at a temperature higher than that of II. The resolution between the carbamate diastereomers was, however, not as good as the diastereomers of II. There was no resolution for the diastereomers of the ethyl isocyanate product.

The advantages of determining optical purity of a chiral compound by using a chiral column is well documented[4–7]. Thus, the use of Chirasil-Val III, a chiral fused-silica capillary column (Alltech), was investigated. The direct approach, without diastereomer formation, via the methyl ester of I, failed to achieve separation between the enantiomers. Acylation or silylation of the methyl ester did not produce resolution of the enantiomers. The diastereomers of the butyl ester, III, and its acyl or silyl derivatives were not resolved on the chiral column. The use of the octyl ester (II), and its acyl or silyl derivatives, has no practical application because of the prolonged retention on the chiral column, operated near the maximum allowable temperature.

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APPENDIX

Derivation of the formula used for the determination of the L *isomer of* L*-Z-oxylysine*

The following symbols are used: D = the D isomer of *Z*-oxylysine; L = the L isomer of *Z*-oxylysine; *d* = the *d* isomer of 2-octanol; *l* = the *l* isomer of 2-octanol; *p* = the fraction of *l* in 2-octanol; *q* = the fraction of L in *Z*-oxylysine.

When *Z*-oxylysine reacts with 2-octanol, four stereoisomers are formed. They are: D*d*, D*l*, L*d* and L*l*. D*d* and L*l* are a pair of enantiomers. D*l* and L*d* are a second pair of enantiomers. Either D*d* or L*l* is a diastereomer of either D*l* or L*d*. Let the total quantity of the four stereoisomers = *T*. Then, the quantity of each stereoisomer can be computed as follows:

$$\text{L}l = qpT \tag{1}$$
$$\text{L}d = q(1 - p)T \tag{2}$$
$$\text{D}l = (1 - q)pT \tag{3}$$
$$\text{D}d = (1 - q)(1 - p)T \tag{4}$$

Note that when the four equations are added, D*d* + D*l* + L*d* + L*l* = *T*, as expected. Note also that the symbols are used to denote the stereoisomers and their respective quantities.

By rearranging eqn. 1 and solving for q:

$$q = \frac{\text{L}l}{pT} \tag{5}$$

In the achiral chromatographic system used, Dd and Ll stereoisomers (enantiomers) are not separated from each other, but as an unresolved peak are separated from the Dl, Ld combination. Thus, the above equation cannot be used as such since one cannot obtain the amount of Ll directly.

Referring to Fig. 1, let area of peak 1 = A_1; area of peak 2 = A_2. When l-2-octanol is reacted with L-Z-oxylysine as described under Experimental, peak 2 is the sole or major peak, depending on the optical purity of the l-2-octanol or the L-Z-oxylysine. Thus, A_1 is the response due to the stereoisomers Dl and Ld. A_2 is the response due to Dd and Ll. Thus, assuming that area is proportional to the quantities of the stereoisomers and the response factors (response/quantity) of the stereoisomers are identical.

$$A_2 = \text{D}d + \text{L}l \tag{6}$$

Note that the proportionality constant that relates area to quantity is assumed to be one for simplicity. Thus,

$$\text{L}l = A_2 - \text{D}d \tag{7}$$

Also,

$$T = A_1 + A_2 \tag{8}$$

Eqn. 5 can now be rewritten as:

$$q = \frac{A_2 - \text{D}d}{p(A_1 + A_2)} \tag{9}$$

The numerator of eqn. 9 can be worked out as follows:

$$\begin{aligned} \text{D}d &= (1 - q)(1 - p)T \\ &= (1 - q)(1 - p)(A_1 + A_2) \end{aligned} \tag{10}$$

Thus $A_2 - \text{D}d$ can be rewritten as:

$$A_2 - [(1 - q)(1 - p)(A_1 + A_2)]$$

i.e.,

$$A_2 - [(1 - p - q + qp)(A_1 + A_2)]$$

i.e.,

$$A_2 - (A_1 + A_2 - pA_1 - pA_2 - qA_1 - qA_2 + qpA_1 + qpA_2)$$

i.e.,

$$A_2 - A_1 - \text{A}_2 + pA_1 + pA_2 + qA_1 + qA_2 - qpA_1 - qpA_2$$

i.e.,

$$pA_1 + pA_2 + qA_1 + qA_2 - qpA_1 - qpA_2 - A_1 \tag{11}$$

The product of the quotient and the denominator of eqn. 9 can be worked out as follows (cross multiplication):

$$qp(A_1 + A_2)$$

i.e.,

$$qpA_1 + qpA_2 \tag{12}$$

Therefore, equating the numerator to the product of the quotient and the denominator,

$$qpA_1 + qpA_2 = pA_1 + pA_2 + qA_1 + qA_2 - qpA_1 - qpA_2 - A_1$$

i.e.,

$$2qpA_1 + 2qpA_2 - qA_1 - qA_2 = pA_1 + pA_2 - A_1$$

i.e.,

$$2qp(A_1 + A_2) - q(A_1 + A_2) = pA_1 + pA_2 - A_1$$

i.e.,

$$(A_1 + A_2)(2qp - q) = pA_1 + pA_2 - A_1$$

i.e.,

$$q(A_1 + A_2)(2p - 1) = p(A_1 + A_2) - A_1 \tag{13}$$

Therefore,

$$q = \frac{p(A_1 + A_2) - A_1}{(A_1 + A_2)(2p - 1)}$$

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