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Resolution of enantiomeric lorazepam and its acyl and O-methyl derivatives and racemization kinetics of lorazepam enantiomers

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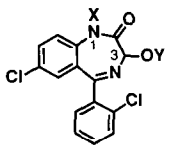
ABSTRACT

Enantiomeric pairs of lorazepam (LZ) and its 3-O-acyl, 1-N-acyl-3-O-acyl, and 3-O-methyl ether derivatives were resolved on high-performance liquid chromatography columns packed with six different chiral stationary phases (CSPs). Resolution was achieved, with several mobile phases of different solvent compositions and with varying chromatograhic resolutions, on at least five of the six CSPs tested. Resolved enantiomers of LZ underwent racemization, whereas enantiomers of 3-O-acyl and 3-O-methyl derivatives were stable. Racemization half-lives of LZ enantiomers were determined by monitoring changes in ellipticity as a function of time. Stability of LZ enantiomers vary substantially depending on the solvents used.

INTRODUCTION

Lorazepam (LZ) is used clinically for the treatment of anxiety and insomnia and has a hydroxyl group at the asymmetric C-3 carbon (see structure in Fig. 1). 3-O-acyl and 3-O-methyl derivatives of LZ are also pharmacologically active in several animals tests [1]. (+)3*S*-Enantiomers of several 1,4-benzodiazepines containing asymmetric carbons at C-3 have been found to possess higher potency in displacing [^{3}H]diazepam (or [^{3}H]flunitrazepam) binding than the respective (−)3*R* enantiomer in synaptosomal preparations from rat cerebral cortex [2–4].

Pirkle and Tsipouras [5] reported the resolution of LZ enantiomers on a column [(*R*)-DNBPG-C] with covalently bonded (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine selectivity, $\alpha = 1.21$); the (+)3*S*-enantiomer was retained more strongly. However,



LZ, X = Y = H
LZA, X = H, Y = $COCH_3$
LZAA, X = Y = $COCH_3$
LZM, X = H, Y = CH_3

Fig. 1. Structures of LZ, LZA, LZAA and LZM.

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LZ enantiomers were not resolved on a column [(S)-DNBL-C] with covalently bonded (*S*)-N-(3,5-dinitrobenzoyl)leucine [5]. Bertucci *et al.* [6] reported that LZ enantiomers can be resolved ($\alpha = 1.59$, the 3*S*-enantiomer was retained more strongly) on a chiral stationary phase (CSP) prepared by anchoring N-methylquininium iodide to γ-mercaptopropylsilanized silica gel.

This paper reports CSP-high-performance liquid chromatographic (HPLC) resolution of enantiomeric pairs of LZ, 3-O-acyl-LZ (LZA), 1-N-acyl-3-O-acyl-LZ (LZAA), and 3-O-methyl-LZ (LZM) (see structures in Fig. 1) with mobile phases of different solvent compositions on six different CSP columns. CSP columns employed were: (1) two covalently bonded (*R*)-DNBPG columns manufactured by different processes, (2) a covalently bonded (*S*)-DNBL-C column, (3) a cellulose triphenylcarbamate ("Chiralcel OC") column, (4) a covalently bonded poly-N-acryloyl-(*S*)-phenylalanine ethyl ester ("Chiraspher") column, and (5) a covalently bonded N-3,5-dinitrophenylaminocarbonyl-(*S*)-valine ("Sumipax OA-3100") column. Half-lives of racemization of LZ enantiomers in a variety of solvents were determined.

EXPERIMENTAL

Materials

LZ[7-chloro-1,3-dihydro-3-hydroxy-5-(*o*-chlorophenyl)-2H-1,4-benzodiazepin-2-one] was generously provided by Dr. Yvon Lefebvre of Wyeth-Ayerst Research (Princeton, NJ, U.S.A.). Molar extinction coefficient of LZ (in methanol) was determined to be 34970 $cm^{-1}\ M^{-1}$ at 230 nm. LZA and LZAA were prepared by reaction of LZ with acetic anhydride in pyridine overnight at room temperature, followed by normal-phase HPLC separation (see below). LZM was converted from LZA in methanol containing 3.4 *M* HCl at 50°C for 40 min, followed by normal-phase HPLC purification. Molar extinction coefficients of LZM, LZA and LZAA, which have UV absorption spectra closely similar to that of LZ, are assumed to be the same as that of LZ.

High-performance liquid chromatography

HPLC was performed using a Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph consisting of a Model 6000A solvent system, a Model M45 solvent delivery system, a Model 660 solvent programmer and a Kratos (Ramsey, NJ, U.S.A.) Model Spectraflow 757 UV–VIS variable-wavelength detector. Samples were injected via a Valco (Houston, TX, U.S.A.) Model N60 loop injector. Retention time and area under chromatographic peaks were determined with a Hewlett-Packard Model 3390A integrator.

Normal-phase HPLC

LZA and LZAA were isolated by normal-phase HPLC on a Zorbax SIL column (25 cm × 6.2 mm I.D., DuPont). The mobile phase was 10% of ethanol–acetonitrile (2:1, v/v) in hexane at a flow-rate of 2 ml/min. Purified LZA and LZAA (racemic as well as enantiomeric) are more stable when the solvents are evaporated and stored at 4°C.

Chiral stationary phase HPLC

Enantiomeric resolutions of LZ, LZA, LZAA and LZM were carried out on six

different CSP columns. These CSP columns are: (1) (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine covalently bonded to spherical particles of 5 μm diameter of γ-aminopropylsilanized silica ["Hi-Chrom Pirkle covalent phenylglycine", abbreviated as (*R*)-DNBPG-C1, 25 cm × 4.6 mm I.D., Regis, Morton Grove, IL, U.S.A.], (2) (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine covalently bonded to spherical particles of 5 μm diameter of γ-aminopropylsilanized silica ("Rexchrom Pirkle covalent D-phenylglycine", abbreviated as (*R*)-DNBPG-C2, 25 cm × 4.6 mm I.D., Regis), (3) (*S*)-N-(3,5-dinitrobenzoyl)leucine covalently bonded to spherical particles of 5 μm diameter of γ-aminopropylsilanized silica [abbreviated as (*S*)-DNBL-C, 25 cm × 4.6 mm I.D., Regis], (4) a column packed with cellulose coated with trisphenylcarbamate (abbreviated as "Chiralcel OC", 25 cm × 4.6 mm I.D., Daicel, Los Angeles, CA, U.S.A.), (5) a column packed with poly-N-acryloyl-(*S*)-phenylalanine ethyl ester covalently bonded to silica gel (abbreviated as "Chiraspher", 25 cm × 4.6 mm I.D., Bodman Chemicals, Stone Mountain, CA, U.S.A.), and (6) a column packed with N-3,5-dinitrophenylaminocarbonyl-(*S*)-valine bonded covalently to silica gel (15 cm × 4.6 mm I.D., abbreviated as "Sumipax OA-3100", Regis). The difference(s) in the manufacturing (*R*)-DNBPG-C1 and (*R*)-DNBPG-C2 columns was not disclosed by Regis. Mobile phases (2 ml/min) were: EA7 and EA8.5 [7% and 8.5% of ethanol–acetonitrile (2:1, v/v) in hexane, respectively]; DC20P*x*[20% dichloroethane, *x*% 2-propanol, and (100 − 20 − x)% hexane]; DC20E*x*[20% dichloroethane, *x*% ethanol, and (100 − 20 − x)% hexane].

Kinetics of racemization

Within 30 s of separation by CSP-HPLC, changes of ellipticity ($\Delta\Phi$) of an enantiomeric LZ(0.6 to 1.5 A_{230} per ml of HPLC elution solvent) were recorded at 255 or 260 nm (at or near the peak of a Cotton effect) as a function of time in a thermostated quartz cuvette [7,8]. Because LZ does not absorb above 360 nm and for the purpose of reducing the monitoring time, ellipticity at 370 nm was used as the baseline for a completely racemized sample. The half-life of racemization ($t_{1/2}$) was determined by plotting log ($\Delta\Phi$) versus time. Racemization of enantiomeric LZ in all solvents tested follows first-order kinetics. Organic solvents for determining $t_{1/2}$ are the mobile phases used in CSP-HPLC. For the determination of racemization $t_{1/2}$ in aqueous solutions and protic organic solvents such as methanol, ethanol and 2-propanol, LZ enantiomers were resolved using solvent DC20P5 (solvent mixtures are described in the section above) on the Chiralcel OC column. Solvent was evaporated by a stream of nitrogen and the LZ enantiomer was redissolved in either an aqueous solution or an alcohol just prior to monitoring changes in ellipticity as described above.

Spectral analysis

Mass spectral analysis was performed on a Finnigan 4000 gas chromatograph–mass spectrometer with a Technivent 1050 data system. Samples were introduced by a Vacumetrics DCI desorption probe with a 150°C ionizer temperature in either the electron impact (EI) mode at 70 eV or chemical ionization (CI, NH_3) mode. UV–VIS absorption spectra of samples (in methanol) were determined using a 1-cm path length quartz cuvette with a Cary 118C (Varian, Palo Alto, CA, U.S.A.) spectrophotometer. Circular dichroism (CD) spectra of samples (in acetonitrile) in a quartz cell of 1 cm

path length were measured at ambient temperature with a Jasco 500A spectropolarimeter equipped with a Model DP500 data processor. The concentration of the sample is indicated by $A_{\lambda 2}$/ml (absorbance units at wavelength $\lambda 2$ per ml of solvent). CD spectra are expressed by ellipticity ($\Phi_{\lambda 1}/A_{\lambda 2}$, in millidegrees) for solutions that have an absorbance of $A_{\lambda 2}$ unit per ml of solvent at wavelength $\lambda 2$ (usually the wavelength of maximal absorption). Under conditions of measurements indicated above, the molar ellipticity ($[\theta]_{\lambda 1}$, in degrees cm^2 dmole^{-1}) and ellipticity ($\Phi_{\lambda 1}/A_{\lambda 2}$, in millidegrees) are related to the extinction coefficient ($\varepsilon_{\lambda 2}$, in cm^{-1} M^{-1}) as follows:

$$[\theta]_{\lambda 1} = 0.1\,\varepsilon_{\lambda 2}(\Phi_{\lambda 1}/A_{\lambda 2})$$

RESULTS AND DISCUSSION

Preparation of LZ derivatives

Acetylation of LZ in pyridine with acetic anhydride produced a monoacetate LZA (M^+ at m/z 362/364 with fragment ions at m/z 320/322, 291/293 and 275/277; EI) and a diacetate LZAA ($M^+ + 1$ at m/z 404/406 with fragment ions at m/z 363/365, 337/339, 320/322, 305/307 and 295/297; CI, NH_3). A minor unknown product was eluted immediately after LZAA (Fig. 2). The 3-O-methyl product LZM (M^+ at m/z 355; EI) was converted from LZA in HCl–methanol and purified by normal-phase HPLC (retention time 18.5 min in Fig. 2). LZA slowly underwent spontaneous hydrolysis in solution to form LZ. LZAA was slowly hydrolyzed in solution to form

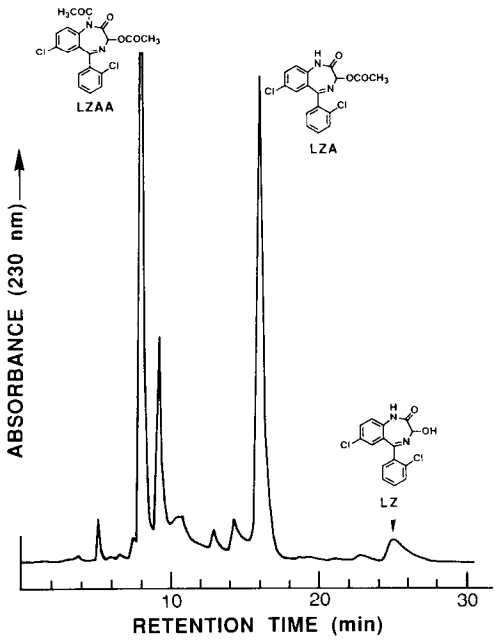

Fig. 2. Normal-phase HPLC separation of LZAA, LZA and LZ. Column, Zorbax SIL (250 mm × 6.2 mm I.D.); mobile phase, 10% ethanol–acetonitrile (2:1) in hexane; flow-rate, 2 ml/min. Under the same chromatographic conditions, LZM has a retention time of 18.5 min.

LZA and LZ. Both LZA and LZAA were more stable at 4°C following removal of solvent.

CSP-HPLC resolution and absolute configuration of enantiomers

The enantiomeric pairs of LZ, LZA, LZAA and LZM were separated on six different CSPs, with varying chromatographic resolutions (Table I). Several mobile phases containing various proportions of the following solvents were used: 2-propanol–hexane, ethanol–acetonitrile–hexane, dichloroethane–2-propanol–hexane, dichloroethane–ethanol–hexane. Consistent with an earlier report on the enantiomeric resolution of oxazepam and its derivatives [7], replacing 10% of 2-propanol in hexane with 7–10% of ethanol–acetonitrile (2:1, v/v) in hexane reduced the retention times of enantiomers and improved the efficiency of enantiomeric separation. The elution order of enantiomeric pairs was established by CD spectral analysis. It is known that the enantiomers of oxazepam, LZ and other 3-substituted 1,4-benzodiazepines that are more strongly retained on the (*R*)-DNBPG-C1 column have positive signs of optical rotations at 589 nm [5]. Furthermore, the (+)-oxazepam has the 3*S* absolute stereochemistry [5]. The 3*R*-enantiomers of many 3-substituted 1,4-benzodiazepines including oxazepam and LZ all have negative signs of CD Cotton effects at *ca.* 255 nm [6–11]. Based on the available information [5–11], the relationship between elution order on CSP-HPLC, sign of optical rotation at 589 nm, CD spectrum, and absolute configuration of LZ enantiomers is therefore established. The CD spectrum of the 3*R*-LZ less strongly retained on (*R*)-DNBPG-C1 is shown in Fig. 3. The enantiomers of LZA, LZAA and LZM that have CD spectra similar to that of 3*R*-LZ are assigned to have the 3*R* absolute stereochemistry (Fig. 3).

The major observations of the enantiomeric resolution of LZ, LZA, LZAA and LZM (Table I) are summarized below:

(1) The enantiomers of LZ were separated on both (*R*)-DNBPG-C1 and (*R*)-DNBPG-C2, but were not separated on (*S*)-DNBL-C. These results are consistent with those reported by Pirkle and Tsipouras [5], who used 10% of 2-propanol in hexane as the mobile phase.

(2) According to Regis, (*R*)-DNBPG-C1 and (*R*)-DNBPG-C2 are prepared by different processes. Retention times of enantiomers on (*R*)-DNBPG-C2 were longer than on (*R*)-DNBPG-C1. However, the enantiomeric pairs were resolved with comparable resolution values.

(3) The enantiomeric pairs of LZA, LZAA, and LZM were efficiently resolved on (*S*)-DNBL-C, while LZ enantiomers could only be resolved with resolution value $\leqslant 0.1$ (Table I). Pirkle and Tsipouras [5] have proposed that the hydrogen at the N1 amide nitrogen of 3-substituted 1,4-benzodiazepines is important for the chiral recognition responsible for the resolution of enantiomers. Since the enantiomers of LZ were not resolved on the (*S*)-DNBL-C, it appears that LZ is an exception to the chiral recognition mechanism proposed [5]. Both LZA and LZM have a free hydrogen at the N1 amide nitrogen. Unlike those of LZ, the enantiomeric pairs of LZA and LZM were efficiently resolved. Substitution of the N1 amide hydrogen of LZA with an acyl group did not reduce the separability of LZAA enantiomers. Thus the chiral recognition mechanism proposed for the (*R*)-DNBPG-C in the enantiomeric resolution of 3-substituted 1,4-benzodiazepines [5] is not applicable for the (*S*)-DNBL-C. It is also interesting to note that, substitution of the N1 amide hydrogen of LZA with an acyl group did not reduce the separability of LZAA enantiomers on (*R*)-DNBPG-C.

TABLE I

CSP-HPLC RESOLUTION OF ENANTIOMERIC LZ, LZA, LZAA and LZM

See text for the assignment of absolute configurations of resolved enantiomers. Enantiomers are designated by E_1 and E_2 according to their elution order. CSPs are described in the Experimental section. Eluents EA7 and EA8.5 are 7% and 8.5% of ethanol–acetonitrile (2:1, v/v) in hexane, respectively. Eluent P10 is 2-propanol–hexane (9:1, v/v). Eluents DC20P*x* are 20% dichloroethane, *x*% 2-propanol and (100−20−*x*)% hexane. Eluents DC20E*x* are 20% dichloroethane, *x*% ethanol and (100−20−*x*)% hexane. The flow-rate of eluent was 2 ml/min. α and R_s are selectivity and resolution, respectively.

Chemical	CSP	Eluent	Retention Time (min)		α	R_s
			E_1	E_2		
LZ	(*R*)-DNBPG-C1	EA7	30.4 (*R*)	32.5 (*S*)	1.1	1.0
		EA8.5	21.8 (*R*)	23.3 (*S*)	1.1	1.1
		DC20P5	22.5 (*R*)	26.2 (*S*)	1.2	1.5
		P10	40.4 (*R*)	47.3 (*S*)	1.2	0.9
	(*R*)-DNBPG-C2	EA8.5	35.9 (*R*)	39.0 (*S*)	1.1	1.3
		DC20P5	66.2 (*R*)	71.1 (*S*)	1.2	0.8
	(*S*)-DNBL-C	EA8.5	21.0 (*R*)	21.2 (*S*)	1.0	*ca.* 0.1[a]
		DC20P6	25.5	25.5	1.0	0
	Chiralcel OC	DC20P5	21.0 (*R*)	37.4 (*S*)	1.9	4.7
		DC20P8	13.5 (*R*)	23.3 (*S*)	1.9	3.9
	Chiraspher	EA7	40.0 (*S*)	41.7 (*R*)	1.1	0.7
		DC20P6	31.6 (*S*)	35.5 (*R*)	1.1	1.7
	Sumipax OA-3100	DC20E7	21.1 (*S*)	24.3 (*R*)	1.2	1.0
		DC20P10	21.3 (*S*)	24.6 (*R*)	1.2	0.9
LZA	(*R*)-DNBPG-C1	EA7	15.8 (*R*)	17.2 (*S*)	1.1	1.6
		EA8.5	14.4 (*R*)	15.8 (*S*)	1.1	1.8
		DC20P4	10.9 (*R*)	13.4 (*S*)	1.3	2.0
	(*R*)-DNBPG-C2	EA8.5	17.1 (*R*)	19.0 (*S*)	1.1	1.8
		DC20P4	13.5 (*R*)	17.1 (*S*)	1.3	1.8
	Chiralcel OC	DC20P5	10.0	10.0	1.0	0
	(*S*)-DNBL-C	EA7	15.9 (*S*)	17.6 (*R*)	1.1	1.9
		EA8.5	10.2 (*S*)	10.7 (*R*)	1.1	0.8
		DC20P3	18.0 (*S*)	22.3 (*R*)	1.3	2.2
	Chiraspher	DC20P5	12.5 (*S*)	14.0 (*R*)	1.1	1.4
	Sumipax OA-3100	DC20E5	7.3 (*R*)	8.6 (*S*)	1.3	1.3
LZAA	(*R*)-DNBPG-C1	EA5	22.3 (*R*)	24.6 (*S*)	1.1	1.5
		EA8.5	14.2 (*R*)	15.6 (*S*)	1.1	1.8
		DC20P4	11.4 (*R*)	13.8 (*S*)	1.3	1.8
	(*R*)-DNBPG-C2	EA8.5	16.8 (*R*)	18.6 (*S*)	1.1	1.8
		DC20P4	13.0 (*R*)	16.4 (*S*)	1.3	2.2
	(*S*)-DNBL-C	EA7	16.1 (*S*)	17.9 (*R*)	1.1	1.6
		EA8.5	11.2 (*S*)	12.1 (*R*)	1.1	0.8
		DC20P3	17.8 (*S*)	22.1 (*R*)	1.3	2.0
	Chiralcel OC	DC20P3	17.8	17.8	1.0	0
	Chiraspher	DC20P5	12.6 (*S*)	14.1 (*R*)	1.1	1.4
	Sumipax OA-3100	DC20E3	12.8 (*R*)	14.3 (*S*)	1.2	0.8
LZM	(*R*)-DNBPG-C1	EA7	26.2 (*R*)	28.3 (*S*)	1.1	1.3
		EA8.5	21.0 (*R*)	23.2 (*S*)	1.1	2.1
		DC20P4	27.9 (*R*)	33.3 (*S*)	1.2	1.7
	(*R*)-DNBPG-C2	EA8.5	30.4 (*R*)	33.1 (*S*)	1.1	1.9
		DC20P4	38.0 (*R*)	57.5 (*S*)	1.6	1.5
	(*S*)-DNBL-C	EA7	25.3 (*S*)	27.3 (*R*)	1.1	1.4
		DC20P3	49.9 (*S*)	55.9 (*R*)	1.1	1.0
	Chiralcel OC	DC20P5	12.4 (*S*)	14.4 (*R*)	1.2	1.0
	Chiraspher	DC20P6	13.9 (*S*)	19.6 (*R*)	1.5	4.2
	Sumipax OA-3100	DC20E8	17.5 (*R*)	18.4 (*S*)	1.1	0.3

[a] Elution order of enantiomers was established by CD spectral measurement of the front one-third and back one-third of the chromatographic peak.

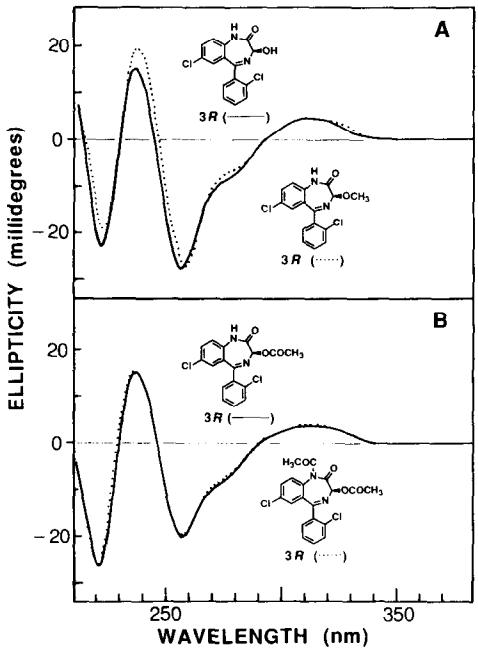

Fig. 3. CD spectra of the 3*R*-enantiomers of LZ(A), LZM(A), LZA(B) and LZAA(B) in acetonitrile. Enantiomers of LZ were separated on Chiralcel OC with eluent DC20P5 (this eluent minimizes the racemization following enantiomeric separation; see racemization $t_{1/2}$ in Table II). The characteristic CD Cotton effects are: 3*R*-LZ (enantiomeric excess, ee $\geqslant$ 95%), $\Phi_{255}/A_{227} = -27.8$ millidegrees; 3*R*-LZM (*ca.* 99% ee), $\Phi_{257}/A_{229} = -27.3$ millidegrees; 3R-LZA (*ca.* 99% ee), $\Phi_{257}/A_{229} = -20.2$ millidegrees; 3*R*-LZAA, 3*R*-LZM (*ca.* 96% ee), $\Phi_{257}/A_{229} = -19.6$ millidegrees.

(4) The most efficient separation of LZ enantiomers ($R_s \geqslant 3.9$) was achieved with the Chiralcel OC column. The Chiralcel OC column also afforded the separation of LZM enantiomers ($R_s = 1.0$), but not the enantiomeric pairs of LZA and LZAA ($R_s = 0$).

(5) Enantiomeric pairs of LZ, LZA, LZAA and LZM were all resolved on the Chiraspher and Sumipax OA-3100 columns, with R_s ranging from 0.3 to 4.2.

(6) Elution orders of enantiomeric pairs on various CSPs are indicated in Table I. On the covalently bonded (*R*)-DNBPG columns, the *S*-enantiomers of LZ, LZA, LZAA and LZM were all more strongly retained. On the (*S*)-DNBL-C, the *S*-enantiomer of LZ was more strongly retained, while the *S*-enantiomers of LZA, LZAA and LZM were less strongly retained. The CSP of Chiraspher contained (*S*)-phenylalanine. The *R* enantiomers of LZ, LZA, LZAA and LZM were all more strongly retained on Chiraspher. In comparison, the CSP of Sumipax OA-3100 contains (*S*)-valine. However, the *R* enantiomers of LZ was more strongly retained, while the *R* enantiomers of LZA, LZAA and LZM were all less strongly retained on Sumipax OA-3100. On the Chiralcel OC column, enantiomeric pairs of LZ and LZM were resolved with different elution order; enantiomeric pairs of LZA and LZAA were not resolved.

(7) Enantiomeric pairs of LZ and LZA can be separated by a single

chromatographic run on either (*R*)-DNBPG-C1 or (*R*)-DNBPG-C2. This observation has been applied successfully as a simple and sensitive analytical method in the analysis of hydrolysis products of racemic LZA by esterases (unpublished data). In two earlier reports [7,12], an (*S*)-DNBL-C column was used successfully to analyze samples resulting from enantioselective hydrolysis of racemic 3-O-acyloxazepam by esterases prepared from the livers and brains of rats and from human livers.

Kinetics of racemization of LZ enantiomers

Enantiomers of LZ readily undergo racemization in a variety of solvents. We have recently developed a method to determine racemization half-lives of enantiomers of several 3-hydroxylated 1,4-benzodiazepines [7,8,11]. Kinetics of racemization of LZ enantiomers in various solvents were determined (Table II). The solvents were mostly the mobile phases used in CSP-HPLC separation of enantiomers. Enantiomeric LZ have a fairly long racemization $t_{1/2}$ (88 min, Table II) in solvent DC20P5. Hence, for determining racemization $t_{1/2}$ in aqueous solutions and protic organic solvents, LZ enantiomers were obtained using solvent DC20P5 on Chiralcel OC column. Evaporation of solvent DC20P5 did not cause significant racemization of LZ enantiomers.

As shown in Table II, increase of the percentages of 2-propanol in solvents DC20P*x* (x = 5 to 10% of 2-propanol) results in the decrease of racemization $t_{1/2}$. Thus the rate of racemization is progressively faster in solvents of increasing polarity. Replacing 2-propanol with ethanol (or a mixture of ethanol and acetonitrile) in solvents DC20P*x* (x = 5 to 10% of 2-propanol) significantly reduces racemization $t_{1/2}$. Racemization $t_{1/2}$ (min) in several pure organic solvents are: methanol (9.2), ethanol (14.3), 2-propanol (33.0), dichloroethane (> 5000) and acetonitrile (> 5000). These results suggest that LZ enantiomers are more stable (against racemization) in an

TABLE II

RACEMIZATION HALF-LIVES OF ENANTIOMERIC LZ IN VARIOUS SOLVENTS

3*R*-LZ and 3*S*-LZ have identical racemization $t_{1/2}$. Solvents EA7 and EA8.5 are 7% and 8.5% of ethanol–acetonitrile (2:1, v/v) in hexane, respectively. Solvents DC20P*x* are 20% dichloroethane, x% 2-propanol and $(100-20-x)$% hexane. Solvents DC20E*x* are 20% dichloroethane, x% ethanol and $(100-20-x)$% hexane. $t_{1/2}$ determined as previously described [7,8,11].

Solvent	Temperature (°C)	$t_{1/2}$ (min)
0.1 *M* Tris–HCl, pH 7.5	37	1.3
0.1 *M* Tris–HCl, pH 7.5	23	5.0
Methanol	23	9.2
Ethanol	23	14.3
2-Propanol	23	33.0
EA7	23	26.2
EA8.5	23	23.0
DC20E7	23	27.5
DC20P10	23	55.0
DC20P8	23	75.5
DC20P6	23	81.0
DC20P5	23	88.0
Dichloroethane	23	> 5000[a]
Acetonitrile	23	> 5000[a]

[a] No detectable change in ellipticity for 3–6 hours of monitoring.

aprotic environment. Decrease in temperature also stabilizes the LZ enantiomers. These results are consistent with those of an earlier study on the stability of oxazepam enantiomers [7]. Enantiomers of LZA, LZAA and LZM did not undergo racemization in the solvents studied. Enantiomers of other 3-hydroxy-1,4-benzodiazepines such as temazepam, lormetazepam, 3-hydroxyprazepam and 3-hydroxyhalazepam also undergo racemization with various racemization $t_{1/2}$ [7,8,11].

Racemization of oxazepam enantiomers was proposed to be due to equilibrium with a tautomer with an open aldehyde form [13,14]. Similar mechanism of racemization probably also operates in the racemization of enantiomeric LZ and other 3-hydroxy-1,4-benzodiazepines. It appears that the open aldehyde tautomers of 3-hydroxy-1,4-benzodiazepine enantiomers form more easily as the polarity of solvent increases. Enantiomers of 3-hydroxyprazepam (with a cyclopropylmethyl group at N1 of oxazepam) and 3-hydroxyhalazepam (with a trifluoroethyl group at N1 of oxazepam) undergo racemization in 0.1 *M* Tris–HCl (pH 7.5) at 37°C with racemization half-lives of *ca.* 90 and *ca.* 150 min, respectively [8,11]. The racemization half-lives (min) of several other enantiomeric 3-hydroxy-1,4-benzodiazepines in 0.1 *M* Tris–HCl (pH 7.5) at 37°C are: oxazepam (*ca.* 3.0) [8], temazepam (*ca.* 3.4) [8], LZ (*ca.* 1.3) (ref. 8 and Table II), and lormetazepam (*ca.* 0.7) [8]. Thus the enantiomers of 3-hydroxyprazepam and 3-hydroxyhalazepam are considerably more stable than the enantiomeric oxazepam, temazepam, LZ and lormetazepam. These results suggest that it may be possible to substantially stabilize enantiomers of 3-hydroxy-1,4-benzodiazepines such as oxazepam and LZ by substituting either a strong electron-donating group or a strong electron-withdrawing group at N1 position. Substitution at aromatic ring positions and introduction of steric factors into the molecule may also influence the stability of enantiomers.

CONCLUSIONS

Enantiomers of LZ, LZA, LZAA and LZM can be separated with various chromatographic resolution values by HPLC using several different types of CSP columns with a variety of mobile phases. LZ enantiomers undergo facile racemization and the rates of racemization in organic and aqueous solvents can be determined by monitoring changes in ellipticity as a function of time with a spectropolarimeter. LZ enantiomers can be stabilized in aprotic solvents and other media of low polarity.

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