Liquid Chromatographic Resolution of Racemic 1,4-Benzodiazepin-2-ones on a Chiral Stationary Phase Based on *N,N'*-Bis-(3,5-dinitrobenzoyl)-2,3-diamino-1,4-butandiol

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Enantiomers of a series of 3-substituted 1,4-benzodiaepin-2-ones such as camazepam, lorazepam, lormetazepam and oxazepam, which belong to a class of widely used anxiolytics and/or tranquilizer,[1] have been known to show different pharmacological activity in our body.[2] Consequently, exact determination of the enantiomeric composition of 3-substituted 1,4-benzodiaepin-2-ones is very important. Among various methods, liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) are generally known as the most accurate, convenient and economic means for the determination of enantiomeric composition of chiral compounds and, consequently, various CSPs have been developed.[3] Some CSPs based on cellulose derivatives and β-cyclodextrins and Pirkle-type CSPs have been applied to the resolution of 3-substituted 1,4-benzodiaepin-2-ones.[4] Especially, Pirkle-type CSP based on (*S*)-*N*-(3,5-dinitrobenzoyl)leucine were quite successful in the resolution of 3-substituted 1,4-benzodiaepin-2-ones.[5]

Recently, we developed a new Pirkle-type CSP (CSP **1**, Figure 1) based on (2*S*,3*S*)-*O,O'*-bis-(10-undecenoyl)-*N,N'*-bis-(3,5-dinitrobenzoyl)-2,3-diamino-1,4-butandiol.[6] CSP **1** was successfully applied to the resolution of racemic anilide derivatives of *N*-acetyl-α-amino acids, 1,1'-bi-2-naphthol, 3,3'-diaryl-1,1'-bi-2-naphthols and some chiral drugs including a diuretic, bendroflumethiazide, and non-steroidal anti-inflammatory agents such as naproxen and alminoprofen. However, CSP **1** has not been applied to the resolution of 3-substituted 1,4-benzodiaepin-2-ones. In this study, we wish to extend the use of CSP **1** to the resolution of 3-substituted 1,4-benzodiaepin-2-ones.

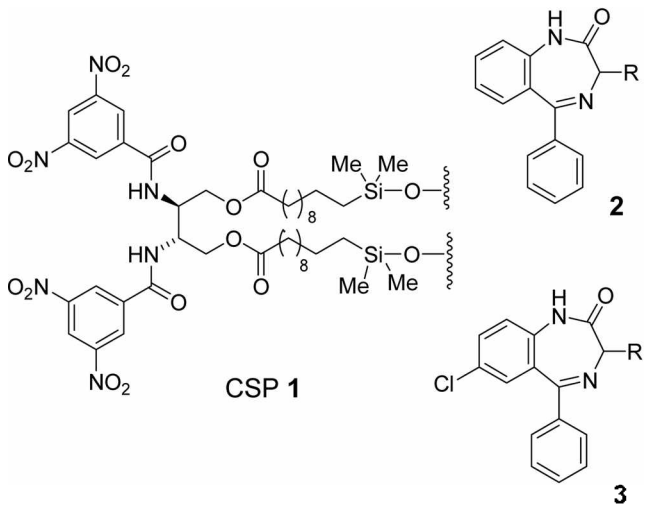

Figure 1. Structures of CSP **1** and 3-substituted 1,4-benzodiazepin-2-ones **2** and **3**.

Two types of 3-substituted 1,4-benzodiaepin-2-ones (**2** and **3**, Figure 1) were used for the enantioseparation on CSP **1** in this study. On Pirkle-type CSPs, mixtures of 2-propanol or ethanol in hexane have been usually used as mobile phases.[7] As an effort to find out which alcohol is better between ethanol and 2-propanol as an alcohol component in mobile phase for the resolution of 3-substituted 1,4-benzo-

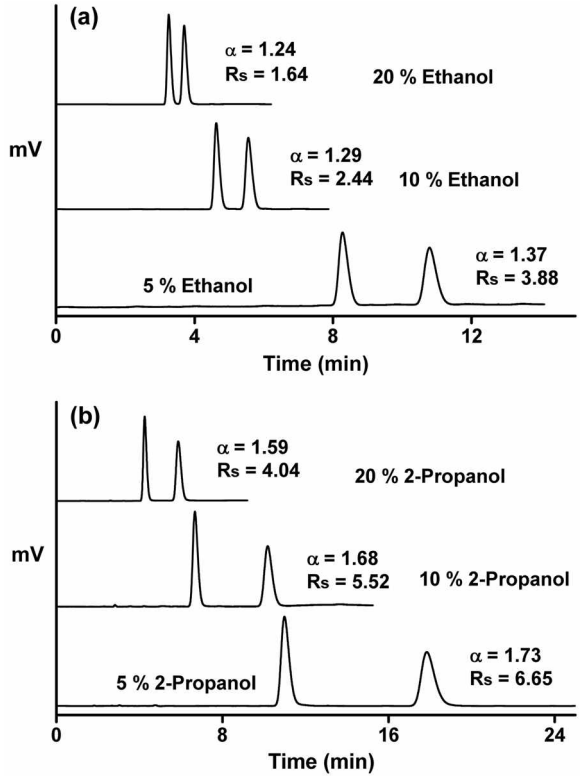

Figure 2. Chromatograms for the resolution of 3-isobutyl-1,3-dihydro(2H)-5-phenyl-1,4-benzodiazepin-2-one **2e** with the variation of the content of (a) ethanol or (b) 2-propanol in hexane as a mobile phase. Flow rate: 2.0 mL/min. Detection: 254 nm UV. Column temperature: 20 °C.

Table 1. Resolution of 3-substituted 1,4-benzodiazepin-2-ones (**2** and **3**) on CSP **1** with the variation of the content of 2-propanol in hexane[a]

Analyte		5% 2-Propanol in hexane			10% 2-Propanol in hexane			20% 2-Propanol in hexane		
	R	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
2a	CH_3	8.05 (S)	1.37	3.82	4.32 (S)	1.35	3.27	2.26 (S)	1.30	2.37
2b	$(CH_2)_3CH_3$	6.05 (S)	1.59	5.41	3.03 (S)	1.56	4.95	1.76 (S)	1.47	3.41
2c	$(CH_2)_2SCH_3$	9.88 (S)	1.45	3.94	5.31 (S)	1.42	3.88	2.82 (S)	1.36	2.00
2d	$CH(CH_3)_2$	5.15 (S)	1.74	5.89	2.62 (S)	1.68	4.86	1.56 (S)	1.59	3.60
2e	$CH_2CH(CH_3)_2$	6.04 (S)	1.73	6.65	3.50 (S)	1.68	5.52	1.86 (S)	1.59	4.04
2f	$CH_2C_6H_5$	12.59 (S)	1.26	2.95	6.91 (S)	1.24	2.35	3.60 (S)	1.21	1.75
3a	CH_3	5.99 (S)	1.22	2.47	3.35 (S)	1.21	1.83	1.80 (S)	1.18	1.42
3b	$(CH_2)_3CH_3$	4.58 (S)	1.38	3.96	2.19 (S)	1.43	3.37	1.39 (S)	1.30	1.85
3c	$(CH_2)_2SCH_3$	7.59 (S)	1.29	2.59	4.20 (S)	1.26	2.36	2.27 (S)	1.22	1.64
3d	$CH(CH_3)_2$	3.89 (S)	1.47	4.26	2.53 (S)	1.35	2.92	1.23 (S)	1.37	2.23
3e	$CH_2CH(CH_3)_2$	3.86 (S)	1.47	4.10	2.65 (S)	1.47	3.70	1.46 (S)	1.40	2.68
3f	$CH_2C_6H_5$	9.64 (S)	1.09	1.05	5.26 (S)	1.08	0.73	2.88 (S)	1.04	

[a]Flow rate: 2.0 mL/min. Detection: 254 nm UV. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. Absolute configuration of the first eluted enantiomers is included in the parenthesis. α: Separation factor. R_S: Resolution factor.

diaepin-2-ones on CSP **1**. 3-isobutyl-1.3-dihydro(2H)-5-phenyl-1.4-benzodiazepin-2-one **2e** was resolved on CSP **1** with the variation of the content of ethanol or 2-propanol in hexane and the resulting chromatograms are illustrated in Figure 2. As shown in Figure 2. both of the separation (α) and the resolution factor (R_S) are greater when 2-propanol in hexane was used as a mobile phase. From these results, we conclude that 2-propanol is better than ethanol as an alcohol component in mobile phase.

Chromatographic resolution of 3-substituted 1,4-benzodiaepin-2-ones (**2** and **3**) on CSP **1** was performed by using mobile phases consisting of 2-propanol in hexane. The chromatographic resolution results are summarized in Table 1. As shown in Table 1. the separation and the resolution factors always increase except for the separation factor for the resolution of analyte **3b** as the content of 2-propanol in hexane is decreased. In the case of analyte **3b**, the separation factor shows the maximum value when 10% 2-propanol in hexane is used as a mobile phase. Even though most analytes are resolved quite well under every mobile phase condition. analyte **3f** was resolved quite poorly. the resolution factor being less than 1.00 when 20% or 10% 2-propanol in hexane was used as a mobile phase. However. the baseline resolution of analyte **3f** was observed when 5% 2-propanol in hexane was used as a mobile phase. From these results. it is concluded that the best mobile phase condition for the resolution of 3-substituted 1.4-benzodiaepin-2-ones on CSP **1** is. in general. 5% 2-propanol in hexane in terms of the separation and the resolution factors. However. it should be noted that 20% 2-propanol in hexane is an attractive mobile phase condition especially for the analytical purpose except for the resolution of analyte **3f** in terms of reducing analytical time and saving solvent.

Between the two types of analytes. type **2** analytes are always resolved better and retained longer than type **3** analytes as shown in Table 1. For the chiral recognition of 3-substituted 1.4-benzodiaepin-2-ones on a Pirkle-type CSP derived from (*S*)-(*N*-3.5-dinitrobenzoyl)leucine. π-π donor-acceptor interaction between the 3.5-dinitrobenzoyl group of the CSP and the benzo ring of the analytes has been proposed to play an essential role.[5a] Similarly. π-π donor-acceptor interaction between the π-acidic 3.5-dinitrobenzoyl group of CSP **1** and the π-basic benzo ring of the analytes seems to be very important for the resolution of analytes **2** and **3**. The effectiveness of π-π donor-acceptor interaction between the 3.5-dinitrobenzoyl ring of the CSP and the benzo ring of the analytes might be dependent on the strength of the π-basicity of the benzo ring of the analytes. Between the non-substituted benzo ring of analytes **2** and the chlorine substituted benzo ring of analytes **3**. the former is more π-basic and consequently. analytes **2** should be retained longer than analytes **3**. The more effective π-π donor-acceptor interaction between the 3,5-dinitrobenzoyl ring of the CSP and the benzo ring of analytes **2** might be also responsible for the higher enantioselectivity denoted by the separation factors (α).

In summary. CSP **1** was applied to the resolution of 3-substituted 1.4-benzodiaepin-2-ones. The resolution was quite successful especially when 5% 2-propanol in hexane was used as a mobile phase. Between the two types of analytes. analytes **2**. which contain relatively more π-basic non-substituted benzo ring. was resolved better in terms of the separation and the resolution factors and retained longer than analytes **3**. which contain relatively less π-basic chlorine-substituted benzo ring. The more effective π-π donor-acceptor interaction between the 3,5-dinitrobenzoyl ring of the CSP and the more π-basic none-substituted benzo ring of analytes **2** was proposed to be responsible for the better resolution and longer retention of analytes **2**.

Experimental Section

Chromatography was performed with an HPLC system consisting of a Waters model 510 pump. a Rheodyne model 7125i injector with a 20 μL sample loop. a YoungLin M720 absorbance detector with a 254 nm UV filter and a Young-

Lin Autochro data Module (Software: YoungLin Autochro-WIN 2.0 plus). The chiral column (250 mm × 4.6 mm I.D. stainless-steel) packed with CSP **1** was available from a prior study.[6] All chromatographic experiments were carried out at a flow-rate of 2.0 mL/min at 20 °C. The chiral column temperature was maintained at 20 °C by using a Julabo F30 Ultratemp 2000 cooling circulator. The void volume was determined by injecting 1,3,5-tri-*tert*-butylbenzene as an unretained analyte. Racemic and optically active 3-substituted 1,4-benzodiazepine-2-ones (**2** and **3**) prepared *via* the known procedure[8] are available from a prior study.[5b] Injection volume of each sample (prepared by dissolving each 3-substituted 1,4-benzodiazepine-2-one in methylene chloride, 1.0 mg/mL) was usually 2 μL. The elution orders were determined by comparing the chromatograms for the racemic and configurationally known samples.

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References and Notes

1. (a) Mennini, T.; Caccia, S.; Garattini, S. *Prog. Drug Res.* **1987**, *31*, 315. (b) Kleemann, A.; Engel, J. *Pharmaceutical Substances*, 3rd ed; Thieme: Stuttgart, 1999.
2. (a) Sunjic, V.; Dejanovic, R.; Palkovic, A.; Klasinc, L.; Kajfez, F. *Tetrahedron Lett.* **1976**, *17*, 4493. (b) Savadori, P.; Bertucci, C.; Domenici, E.; Giannaccini, G. *J. Pharm. Biomed. Anal.* **1989**, *7*, 1735.
3. For example, see (a) *Chiral Separation Techniques: A Practical Approach. Third, Completely Revised and Updated Edition*; Subramanian, G., Ed.; Wiley-VCH: Weinheim, 2007. (b) Hyun, M. H.; Kim, S. N.; Choi, H. J.; Sakthivel, P. *Bull. Kor. Chem. Soc.* **2007**, *28*, 1980.
4. (a) Bertucci, C.; Uccello-Barretta, G.; Savadori, P. *J. Chromatogr.* **1990**, *506*, 617. (b) Katti, A.; Erlandsson, P.; Dappen, R. *J. Chromatogr.* **1992**, *590*, 127. (c) Oliveros, L.; Minguillon, C.; Billaud, C. *J. Pharm. Biomed. Anal.* **1992**, *10*, 925. (d) Lu, X.-L.; Yang, S. K. *J. Chromatogr. A* **1994**, *666*, 249.
5. (a) Pirkle, W. H.; Tsipouras, A. *J. Chromatogr.* **1984**, *291*, 291. (b) Hyun, M. H.; Cho, Y. J.; Kim, J. A. *Bull. Kor. Chem. Soc.* **2005**, *26*, 823.
6. Hyun, M. H.; Boo, C. J.; Choi, H. J.; Kim, Y. K.; Kang, B. S.; Ha, H. J.; Choi, M. K.; Tan, G. *Bull. Kor. Chem. Soc.* **2006**, *27*, 1769.
7. (a) Pirkle, W. H.; Pochapsky, T. C. *Chem. Rev.* **1989**, *89*, 347. (b) Welch, C. J. *J. Chromatogr.* **1994**, *666*, 3. (c) Hyun, M. H.; Cho, Y. J.; Baik, I. K. *Bull. Kor. Chem. Soc.* **2002**, *23*, 1291. (d) Hyun, M. H.; Cho, Y. J.; Choi, H. J.; Lee, K. W. *Bull. Kor. Chem. Soc.* **2004**, *25*, 1977.
8. Sunjic, V.; Kajfez, F.; Stromar, I.; Blazevic, N.; Kolbah, D. *J. Heterocycl. Chem.* **1973**, *10*, 591.