Asian Journal of Chemistry Vol. 22, No. 4 (2010), 2745-2752

Resolution of Diastereomeric Tartaric Acid Monoamides by Reversed-phase High Performance Liquid Chromatography

T. MUNEGUMI*, K. INABA, K. KIKUCHI, Y. TAUCHI† and K. HARADA†
Department of Materials Chemistry and Bioengineering, Oyama National College of Technology, Nakakuki, Oyama, Tochigi-323-0806, Japan
Fax: (81)(285)202880; Tel: (81)(285)202800; E-mail: munegumi@oyama-ct.ac.jp

Diastereomer resolution by high performance liquid chromatography (HPLC) has been useful for determining the correlation between molecular structure and hydrophobicity. Three pairs of diastereomeric tartaric acid monoamides (O,O'-diacetyl-(2R, 3R)-tartaric amides) are resolved into each diastereomer by achiral reversed-phase HPLC (RP-HPLC). There are some correlations between the molecular structure and the elution order of the diastereomers. O,O'-Diacetyl-(2R,3R)-tartaric acid (R)-amides ((R,R,R)-tartaramides) are eluted faster than (R,R,S)-tartaramides. ^{1}H NMR spectra of these compounds in acetonitrile-d^3 (CD_3CN) shows that the dihedral angle is about 110º around the *vicinal* protons of the tartaric acid moiety. When we adopt this value for calculation of the molecular structure, the logarithm of the partition coefficient (log P value) of tartaramides in *n*-octanol per water explains the elution order of diastereomers. The findings play a role in both determination of the elution order and the conformation of similar chiral compounds in RP-HPLC.

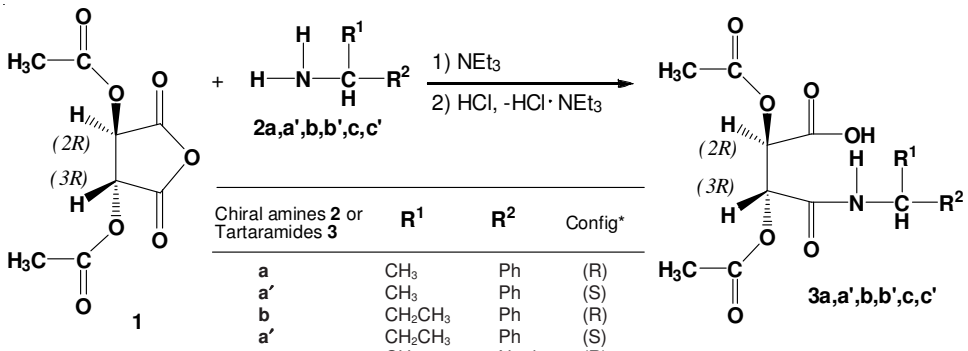
Key Words: Diastereomer, log P, NMR, Semi-empirical calculation, Tartaramide.

INTRODUCTION

High performance liquid chromatography (HPLC)[1,2] is a useful method for determining the absolute configuration of optically active compounds as well as for the preparative-scale separation of such compounds. Direct separation of racemic compounds into the enantiomers has often been performed using chiral stationary phases coated or bonded with chiral selectors[3]. Indirect separation using achiral stationary phases may also be applicable for the separation of diastereomers[3,4], although extra processes are involved to give each enantiomer. However, the latter method using achiral stationary phases[4,5] allows researchers to determine the differences in physical and chemical properties between diastereomers. For example, the hydrophobicity and the conformation of each diastereomer can be determined itself with the achiral reversed-phase columns. Such determinations provide important information that can be used to obtain further improved separation of chiral compounds.

† Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki-305-8571, Japan.

In this paper, the separation of three pairs of diastereomeric monoamides (**3a**, **3a'**, **3b**, **3b'**, **3c**, **3c'**) of O,O'-diacetyl-(2R,3R)-tartaric (Fig. 1) acid using a reversed-phase column, as well as the relationship between the elution order and conformation in solvents of different ratios are described. The three pairs of chiral amines (**2a-c**, **2a'-c'**) have been used for the prediction of stereochemistry in asymmetric induction[6], because comparing the bulkiness of constituent group enables the determination of conformational change.



Chiral amines **2** or Tartaramides **3**	R^1	R^2	Config*
a	CH_3	Ph	(R)
a'	CH_3	Ph	(S)
b	CH_2CH_3	Ph	(R)
a'	CH_2CH_3	Ph	(S)
c	CH_3	Naph	(R)
c'	CH_3	Naph	(S)

* Absolute configurataion of the amine moiety

Fig. 1. Preparation of O,O'-diacetyl-(2R,3R)-tartaric amides

EXPERIMENTAL

^{1}H NMR spectra of tartaric acid monoamides **3a**, **3a'**, **3b**, **3b'**, **3c**, **3c'** were recorded on an EX-270 NMR system (Joel, Tokyo). HPLC analysis was carried out with a Jusco HPLC System (flow pump: 880-PU, detector: 875-UV, recorder: 807-IT).

O,O'-Diacetyl-(2R,3R)-tartaric acid anhydride (**1**) was prepared as described in the literature[7]. Chiral amines (R)-1-phenylethylamine (**2a**) and (S)-1-phenylethylamine (**2a'**) were purchased from Sigma-Aldrich. (R)-1-Phenylpropylamine (**2b**) and (S)-1-phenylpropylamine (**2b'**) were prepared as described in the literatures[8,9]. (R)-1-Naphthylethylamine (**2c**) and (S)-1-naphthylethylamine (**2c'**) were purchased from Sigma-Aldrich. Diastereomeric tartaramides (**3a**, **3a'**, **3b**, **3b'**, **3c**, **3c'**) were prepared by ring-opening of O,O'-diacetyl-(2R,3R)-tartaric acid anhydride (**1**) with the corresponding chiral amines in presence of triethylamine as shown in Fig. 1. After purification of the acidified crude products by silica gel column chromatography, the structures of the compounds **3a**, **3a'**, **3b**, **3b'**, **3c**, **3c'** were determined by IR, NMR and elemental analysis. The elementary analysis agreed with the theoretical data as follows: **3a**: Anal. calcd. for $C_{16}H_{19}NO_7.0.5H_2O$: C, 55.44; H, 5.82; N, 4.04 %. Found: C, 55.31; H, 5.68; N, 3.91 %. **3a'**: Anal. calcd. for $C_{17}H_{19}NO_7.0.5H_2O$: C, 55.44; H, 5.82; N, 4.04 %. Found: C, 55.74; H, 5.59; N, 3.97 %. **3b**: Anal. calcd. for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02; N, 3.99 %. Found: C, 57.87; H, 5.98; N, 3.98 %.

3b': Anal. calcd. for $C_{17}H_{21}NO_7 \cdot 1.5H_2O$: C, 53.95; H, 6.38; N, 3.71 %. Found: C, 53.26; H, 5.64; N, 3.58 %. **3c**: Anal. calcd. for $C_{20}H_{21}NO_7.1.8H_2O$: C, 57.22; H, 5.47; N, 3.32 %. Found: C, 57.07; H, 5.43; N, 3.35 %. **3c'**: Anal. calcd. for $C_{20}H_{21}NO_7.H_2O$: C, 59.29; H, 5.72; N, 3.46 %. Found: C, 59.52; H, 5.77; N, 3.58 %.

High performance liquid chromatography: Acetonitrile and 20 mM sodium phosphate buffer (pH 6.9) were mixed in different ratios (v/v) as the isocratic eluant. The flow rate of eluant was 0.5 mL/min (throughout). A Lichrospher 100 RP-18 column (250 mm × 4.0 mm i.d.) was used for the stationary phase. UV absorbance of the eluted solution was recorded at 230 nm.

log P calculation: Molecular mechanics (MM2) and semi-empirical (MOPAC AM1) calculation of the three pairs of (2R,3R)-tartaramides bearing the fixed dihedral angles in the molecular structure were sequentially carried out using a CAChe work system version 5.0 to give optimized molecular structures. log P values of the tartaramides were determined[10,11] on the basis of the relationship between the calculated log P values and the experimental log P values of authentic compounds.

RESULTS AND DISCUSSION

HPLC Separation: The three pairs of tartaramides were resolved with an isocratic eluant of 20 % acetonitrile (CH_3CN) in aqueous neutral buffer (pH 6.9) as shown in Fig. 2. These tartaramides were completely resolved under the chromatographic conditions used. The tartaramide diastereomers were also resolved using different compositions [15, 25, 30, 40 and 50 % (v/v)] of acetonitrile to the aqueous neutral buffer (pH 6.9). Table-1 shows both of the retention times and the separation factors of these tartaramides. The elution order of the three pairs of tartaramides was as follows: **3a**, **3a'**, **3b**, **3b'**, **3c**, **3c'**. The hydrophobicity order, however, was **3c'**, **3c**, **3b**, **3b'**, **3a**, **3a'**. In RP-HPLC separation system, the retention time is comparable to hydrophobicity.

The bulkiness of the substituent groups attached to the asymmetric carbon of the amine moiety affects the hydrophobicity of the whole molecule, because the groups are all hydrophobic. The order of bulkiness of the groups is naphthyl (Naph) > phenyl (Ph) > ethyl (Et) > methyl (Me) and the order of bulkiness of the tartaramides is **3c = 3c' > 3b = 3b' > 3a = 3a'**. There is no difference in bulkiness between diastereomers. However, there are some differences in hydrophobicity between diastereomeric tartaramides. This is because of the conformational difference depending on the concentration of acetonitrile in the mobile phase.

All (R,R,R)-tartaramides (**3a**, **3b**, **3c**) eluted faster than (R,R,S)-tartaramides (**3a'**, **3b'**, **3c'**) due to their diastereomers under the most conditions. The results shows that the affinity of (R,R,R)-tartaramides to the stationary phase is weaker than that of (R,R,S)-tartaramides. The retention time became shorter and the separation poorer at the higher concentrations of acetonitrile in the eluant, as well as usual HPLC separation using reversed stationary phases. At the highest acetonitrile concentration, the tartaramides did not separate into their diastereomers at all.

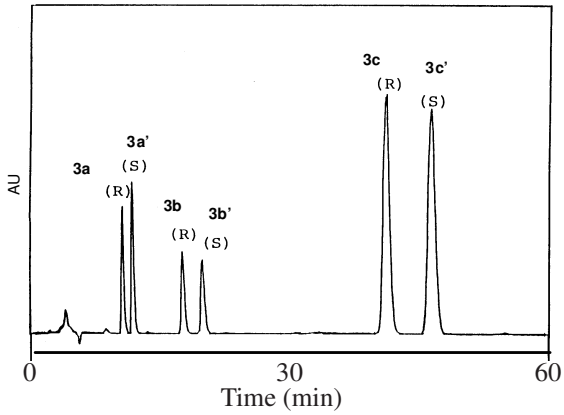


Fig. 2. Resolution of diastereomeric tartaramides using an eluant containing 20 % acetonitrile in pH 6.9 phosphate buffer (v/v) at the flow rate of 0.5 mL/min on Lichrospher RP-230

TABLE-1
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)
SEPARATION OF DIASTEREOMERIC TARTARAMIDES

Diastereomeric tartaramide	Retention time (min)		Separation*	Composition of
	(R,R,R)	(R,R,S)	Factor	CH_3CN (%, v/v)
3a + 3a'	18.25	21.23	1.194	15
	10.63	11.75	1.145	20
	6.34	6.67	1.095	25
	5.23	5.65	1.175	30
	4.15	4.15	1.000	40
3b + 3b'	33.81	40.02	1.201	15
	17.58	19.88	1.156	20
	8.07	8.67	1.115	25
	6.14	6.35	1.066	30
	4.40	4.40	1.000	40
3c + 3c'	119.67	140.0	1.174	15
	41.50	46.83	1.138	20
	13.29	14.46	1.112	25
	8.25	8.67	1.078	30
	4.70	5.11	1.226	40
	4.01	4.01	1.000	50

^{1}H NMR Measurement: To determine the conformation of the tartaramides, ^{1}H NMR of the tartaramides were recorded in CD_3CN or in the mixed solvent of CD_3CN and D_2O using different ratios of D_2O [5, 15 and 25 % (v/v)]. ^{1}H NMR spectra of tartaramides **3a** and **3a'** in CD_3CN (100 %) are shown in Fig. 3. The coupling constant between the two *vicinal* protons at the C2 and C3 positions was 2.64 Hz. This means that the dihedral angle of this moiety is 110º. Table-2 shows the coupling constants due to the tartaramides **3a-c** and **3a'-c'**. The coupling constants for the vicinal protons[12,13] were relevant to their dihedral angles in the Karplus correlation[14] as shown in Fig. 4.

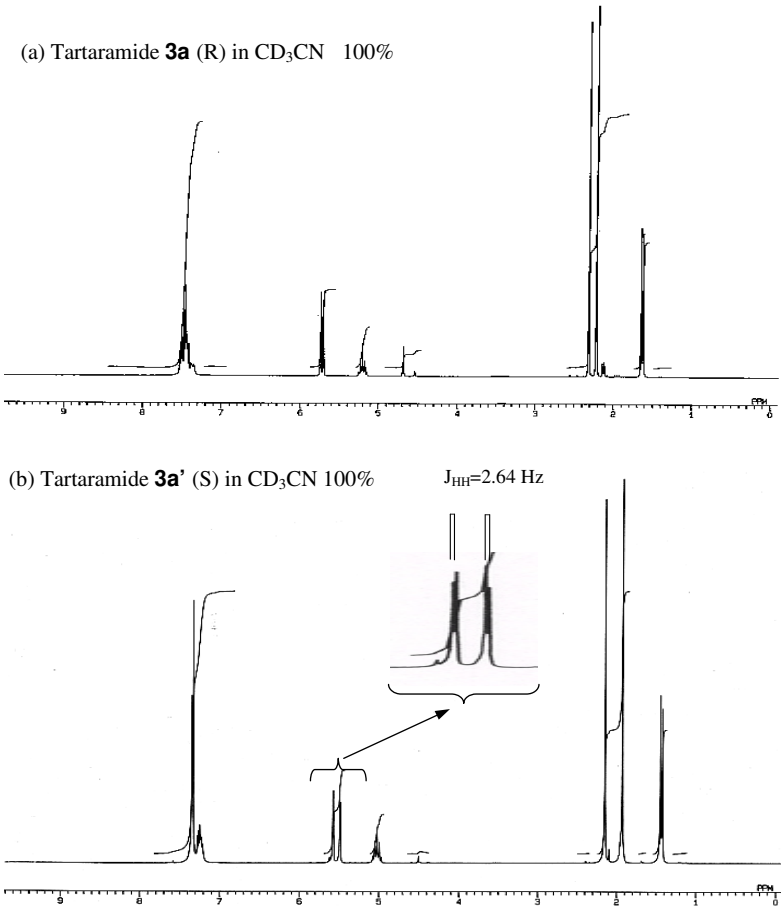


Fig. 3. ^1H NMR spectra of tartaramides **3a** (a) and **3a'** (b) in CD_3CN

TABLE-2
COUPLING CONSTANT OF VICINAL PROTONS

Tartaramide	J-Vales of vicinal protons (Hz)	Estimated dihedral angle (º)
3a	2.64	110 or -110
3a'	2.64	110 or -110
3b	0	85 or -85
3b'	0	85 or -85
3c	2.31	109 or -109
3c'	2.31	109 or -109

The coupling constants for the vicinal protons in solvent CD_3CN (100 %) are also shown in Fig. 3. However, these coupling constants were in the range of 0-2.64 Hz. Such values mean that the dihedral angles are in the range of 85-110º.

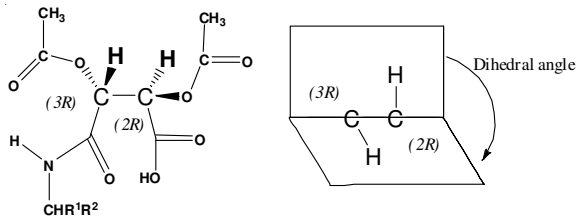


Fig. 4. Dihedral angle between planes of H-C-C and C-C-H in tartaramides

Molecular modeling: Molecular mechanics (MM2) and semi-empirical (AM1) calculations of tartaramides bearing the dihedral angles in CD_3CN lead the conformations as shown in Fig. 5. The conformations in acetonitrile would be comparable with the conformations in the hydrophobic stationary phase such as C18 (octadecyl silyl), because the solute molecules do not form hydrogen bonds with aprotic solvents such as acetonitrile. Calculated log P (C log P) values of the conformers in Fig. 5 were calculated as shown in Table-3. C log P values can be obtained using the following equation[11]:

$$\text{C log P} = -0.33263 - 0.027225*B + 0.048959*C + 0.031116*D - 1.4003*\text{sqrt}(F)) - 1.0241*\text{sqrt}(G) + 1.3851*H$$

In this equation, B: heat of formation in vacuum; C: solvent accessible surface area, D: heat of formation in water, F: number of nitrogen atoms; G: number of oxygen atoms; H: number of nitro groups.

Comparing the C log P values of tartaramides with each other, the values are consistent with the hydrophobicity. The order of largeness of the C log P values is as follows: **3c'** (4.415) > **3c** (4.357) > **3b** (3.943) > **3b'** (3.708) > **3a** (3.390) > **3a'** (3.169). The order is the same as the order of largeness of hydrophobicity determined from RP-HPLC. These results mean that C log P may predict the elution order of diastereomers in RP-HPLC.

TABLE-3
CALCULATED log P VALUES OF THE TARTARAMIDE

Trtaramides	Heat of formation (kcal/mol): B	Solvent accessibility surface area (square angstrom): C	Heat of formation (kcal/mol): D	Molecular formula	Number of nitrogen atom: F	Number of oxygen atom: G	Number of nitro group: H	C log P	Dihedral angle
3a	-272.228	144.125	-307.425	$C_{16}H_{19}NO_7$	1	7	0	3.169	110
3a'	-272.268	149.629	-309.009	$C_{16}H_{19}NO_7$	1	7	0	3.390	110
3b	-276.173	156.123	-312.423	$C_{17}H_{21}NO_7$	1	7	0	3.708	85
3b'	-280.827	161.642	-317.633	$C_{17}H_{21}NO_7$	1	7	0	3.943	85
3c	-253.784	169.984	-293.804	$C_{20}H_{21}NO_7$	1	7	0	4.357	109
3c'	-253.234	169.986	-291.449	$C_{20}H_{21}NO_7$	1	7	0	4.415	109

C log P = -0.33263-0.027225*B + 0.048959*C + 0.031116*D - 1.4003*sqrt(F)) - 1.0241*sqrt(G) + 1.3851*H.

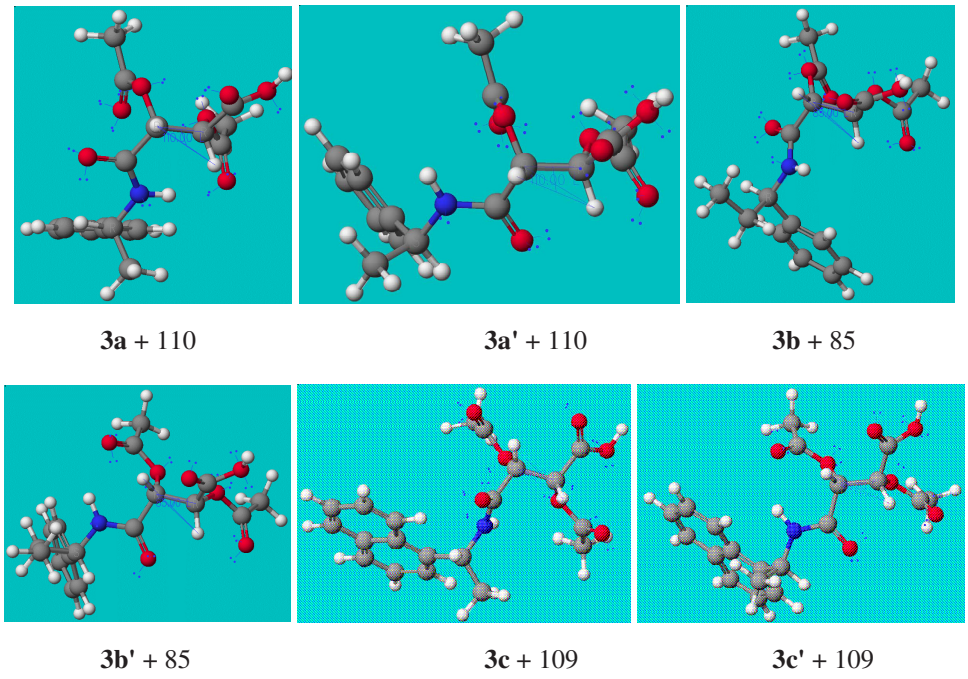


Fig. 5. Calculated conformation of tartaramides **3a-c** and **3'a-c**

Conclusion

The results of this research have demonstrated that three pairs of diastereomeric tartaric acid monoamides (O,O'-diacetyl-(2R,3R)-tartaric amides) were resolved into each diastereomer by achiral reversed-phase HPLC (RP-HPLC). ^{1}H NMR spectra of these compounds in the mixture of acetonitrile-D3 (CD_3CN) showed that the dihedral angle was about 110º around the vicinal protons of the tartaric acid moiety. Molecular mechanics and semiempirical calculations of the tartaramides bearing the dihedral angles in CD_3CN indicate stable conformations and C log P values. The C log P values were consistent with the hydrophobicity. log P calculations based on dihedral angles obtained from NMR measurement may predict the elution order of diastereomers in RP-HPLC.

REFERENCES

1. H.Y. Aboul-Enein and I. Ali, Chiral Separations by Liquid Chromatography and Related Technologies, Marcel Dekker, New York, pp. 9-10 (2003).
2. A.M. Krstulovic, Chiral Separations by HPLC, Ellis Horwood Ltd., Chichester, England (1989).
3. S. Ahuja, Chiral Separations by Chromatography, American Chemical Society, Washington D.C./ Oxford University Press, Oxford, pp. 6-8 (2000).
4. G.K.E. Scriba, Peptide Diastereomers, Separation of, Encyclopedia of Analytical Chemistry, John Wiley and Sons, New York (2006).
5. J. Fekete, M. Milen, L. Hazai, L. Poppe, C.S. Szantay, A. Kettrup and I. Gebefugi, *Chromatographia*, **57**, 147 (2003).

6. K. Harada and T. Munegumi, Comprehensive Organic Synthesis, Vol. 8, B. Trost and I. Fleming, Eds., Pergamon Press, England, pp. 139-158 (1991).
7. A. Wohl and C. Oesterlin, *Chem. Ber*., **34**, 1139 (1901).
8. A.W. Ingersoll, *Org. Synth. Coll.*, **2**, 503 (1943).
9. A.J. Little, J. M'Lean and F.J. Wilson, *J. Chem. Soc*., 336 (1940).
10. N. Bodor, Z. Gabanyi, C.K.A. Wong, *J. Am. Chem. Soc*., **111**, 3783 (1989).
11. T. Munegumi and A. Shimoyama, *Chirality*, **15S**, 108 (2003).
12. M. Karplus, *J. Chem. Phys*., **30**, 11 (1959).
13. M. Karplus, *J. Am. Chem. Soc*., **85**, 2870 (1963).
14. R.M. Silverstein, G.C. Bassler and T.C. Morrill, Spectrometric Identification of Organic Compounds, John Wiley and Sons, New York, edn. 4, p. 210 (1981).

(*Received*: 30 March 2009; *Accepted*: 17 December 2009) AJC-8194