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New Derivatization for Liquid Chromatographic Resolution of Amino Acid Enantiomers

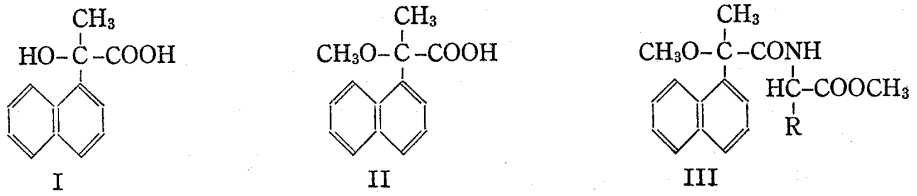
A new method for liquid chromatographic resolution of amino acid enantiomers by the formation of diastereomers has been developed. A chiral reagent used for this purpose, (−)-α-methoxy-α-methyl-1-naphthaleneacetic acid (IIb), was readily prepared by fractionally crystallizing the (+)-α-methylbenzylamine salt. The diastereomers formed from amino acid methyl esters and IIb by the N,N′-dicyclohexylcarbodiimide method were efficiently resolved on the normal phase column.

Keywords——high-performance liquid chromatography; resolution of DL-amino acids; chiral-acylating agent; (−)-α-methoxy-α-methyl-1-naphthaleneacetic acid; diastereomers; normal phase column

In recent years considerable attention has been focused on the chromatographic separation of optical isomers. One of the principal approaches involves the formation of diastereomeric compounds by introducing the second chiral center followed by separation on the conventional phase. Several attempts have been made for optical resolution by high-performance liquid chromatography (HPLC).[1-3] However, they have had only limited use because of their insufficient resolution, sensitivity and versatility. In this communication we wish to report a new method for the liquid chromatographic separation of amino acid enantiomers by converting to the covalently bonded diastereomers.

The design of a promising reagent requires the structural features having both chirality for the efficient resolution and chromophore for the sensitive detection by ultraviolet (UV) monitoring. For this purpose an initial project was directed to the preparation of the Mosher-type[4] naphthalene derivative as a chiral reagent.

The Grignard reaction of pyruvic acid with 1-naphthylmagnesium bromide furnished α-hydroxy-α-methyl-1-naphthaleneacetic acid (I), mp 110—112°, in a fairly good yield. Subsequent methylation with methyl iodide and sodium hydride in dimethylformamide afforded methyl α-methoxy-α-methyl-1-naphthaleneacetate, alkaline hydrolysis of which yielded α-methoxy-α-methyl-1-naphthaleneacetic acid (IIa), mp 161—162°. NMR ($CDCl_3$)δ: 2.01 (3H, s, $-CH_3$), 3.07 (3H, s, $-OCH_3$), 7.30—8.50 (7H, m, Ar–H), 9.45 (1H, s, –COOH). UV λ_{max}^{MeOH} nm (log ε): 263 (3.36), 271 (3.53), 281 (3.57), 287 (3.46), 292 (3.45). The optical isomer was readily resolved by fractionally crystallizing the (+)-α-methylbenzylamine salt from ethanol. This procedure was repeated twice to provide the desired (−)-α-methoxy-α-methyl-1-naphthaleneacetic acid (IIb), mp 111—112°, $[\alpha]_D^{13}$ −106.3° (c=0.16, $CHCl_3$), $[\alpha]_D^{13}$ −128.8° (c=0.10, MeOH), in 41% yield. The optical purity of this chiral reagent was determined to be over 99.5%.



a: (±), b: (−)

Chart 1

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Condensation of IIb with amino acid methyl ester was effected by treatment with N,N′-dicyclohexylcarbodiimide (DCC) in pyridine. The resulting amide (III) was extracted with ethyl acetate, washed and then submitted to HPLC. The reagent showed complete stability to racemization under prolonged reaction conditions. The apparatus used was a Waters Model ALC/GPC 202 R401 high-pressure liquid chromatograph (Waters Associates Inc., Milford) equipped with an UV monitor operated at 280 nm and a μ-Porasil column (1′×1/4″ i.d.). The mobile phase was cyclohexane-ethyl acetate (4: 1—2: 3, v/v) at a flow rate of 0.7 ml/min.

Table I. Separation of Diastereomeric N-(−)-α-Methoxy-α-methyl-1-naphthaleneacetyl Amino Acid Methyl Esters

Amino acid	k'		Relative retention value (α)	Mobile phase[a)]
	D	L		
Alanine	1.91	3.10	1.62	A
Valine	1.76	1.06	1.66	A
Norvaline	2.17	1.22	1.78	A
Leucine	1.83	0.78	2.35	A
Isoleucine	1.53	0.88	1.74	A
Norleucine	1.92	0.96	2.00	A
Proline	1.42	2.38	1.68	A
Phenylglycine	2.56	1.32	1.94	A
Phenylalanine	2.41	1.52	1.59	A
Aspartic acid	5.61	7.00	1.25	A
Glutamic acid	6.46	6.81	1.05[b)]	A
Serine	2.52	7.32	2.90	C
Tyrosine	2.02	1.74	1.16	B
DOPA	2.32	2.04	1.14[b)]	C
Ornithine	2.00	3.52	1.76	D
Lysine	1.60	2.20	1.38	D
Cysteine	2.08	0.98	2.12	C

a) The ratio (v/v) of cyclohexane to ethyl acetate: A) 4:1; B) 2:1; C) 1:1; D) 2:3.
b) The two peaks were not completely resolved.

The chromatographic results on seventeen pairs of enantiomeric amino acid methyl esters are listed in Table I. The k′ and α values refer to the capacity ratio and relative retention value for each pair of diastereomers formed by condensation with (−)-α-methoxy-α-methyl-1-naphthaleneacetic acid. Each amino acid derivative showed a single peak of the theoretical shape on a μ-Porasil column, indicating the excellent chromatographic properties. Almost all the pairs of diastereomers were completely resolved (separation factor, $R>1$)[5] on the normal phase column, but not on the reverse phase column. No marked differences in separation for normal and branched-chain amino acids were observed. The satisfactory resolution was also attained for aromatic and basic amino acids. Although any generalization with regard to the elution order could not be made, it seemed likely that the retention values of L-amino acids were dependent upon their structures, while those of the D-enantiomers were not significantly varied. The retention times of aliphatic L-amino acids were influenced by the alkyl residue, probably due to the change of preferential conformation at the length of two or three carbon chain. The similar chromatographic behaviors on the normal phase column have recently been demonstrated with diastereomers of some isoprenoid acid enantiomers.[6]

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The use of analogous chiral-acylating agents having a fluorophore for chromatographic resolution of amines in small quantities in biological materials will be a subject in the future communication.

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Pharmaceutical Institute
Tohoku University
Aobayama, Sendai

JUNICHI GOTO
MASATOSHI HASEGAWA
SETSUKO NAKAMURA
KAZUTAKE SHIMADA
TOSHIO NAMBARA

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