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NEW DERIVATIZATION REAGENTS FOR THE RESOLUTION OF AMINO ACID ENANTIOMERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A new derivatization procedure has been developed for converting enantiomeric amines into diastereomers for resolution by high-performance liquid chromatography. Two chiral reagents, (—)-α-methoxy-α-methyl-1-naphthaleneacetic acid and (—)-α-methoxy-α-methyl-2-naphthaleneacetic acid, were prepared and optically resolved by fractional crystallization of their (+)-α-methylbenzylamine salts. The diastereomeric amides formed from amino acid methyl esters and (—)-α-methoxy-α-methyl-1-naphthaleneacetic acid by the N,N′-dicyclohexylcarbodiimide method were efficiently resolved on a normal-phase column and responded with satisfactory sensitivity in the ultraviolet detector.

INTRODUCTION

Liquid chromatography has been widely used for the separation of enantiomers. Direct resolution on chiral stationary phases has been carried out with a certain measure of success[1–10]. Alternatively, derivatization of enantiomers with a chiral reagent yields diastereomers that can often be resolved and determined on conventional columns[11–17]. In connection with the pharmacokinetic studies of racemic drugs, a new derivatization reagent that may provide diastereomers more readily distinguishable and responding with higher sensitivity in the UV detector, has been developed. The present paper deals with the synthesis of optically active α-methoxy-α-methyl-1-naphthaleneacetic acid and α-methoxy-α-methyl-2-naphthaleneacetic acid as derivatization reagents, and their applicability to the resolution of enantiomeric amino acid methyl esters by high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Synthesis of derivatization reagents
    α-Hydroxy-α-methyl-2-naphthaleneacetic acid (II). (i) To a solution of 2-

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naphthylglyoxylic acid (I) (2 g), obtainable from 2-acetonaphthone[18], in anhydrous ether (10 ml) was added methylmagnesium iodide in ether (2.2 N) (10 ml). After being stirred for 1 h under ice-cooling, the reaction mixture was acidified with 3 % H_2SO_4 and extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from ether–hexane gave II (1.51 g) as colourless plates (m.p. 155–156°; analysis: calculated for $C_{13}H_{12}O_3$: C, 72.21 %; H, 5.59 %; found: C, 72.03 %; H, 5.57 %).

        (ii) To a stirred solution of magnesium metal (200 mg) in anhydrous ether (8 ml) were added 2-bromonaphthalene (1.5 g) in anhydrous ether (2 ml) and a trace amount of methyl iodide, and the mixture was stirred at room temperature for 1 h under a stream of nitrogen gas. The resulting solution of 2-naphthylmagnesium bromide (III) was slowly added to a solution of pyruvic acid (330 mg) in ether (4 ml) and stirred at room temperature for 30 min. The reaction mixture was acidified with 3 % H_2SO_4 and extracted with ethyl acetate. The organic phase was washed with water and extracted with 5 % Na_2CO_3. The aqueous layer was then acidified with 5 % HCl and reextracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from ether–hexane gave II (400 mg) as colourless plates (m.p. 155–156°). A mixed melting point determination on admixture with the sample obtained from procedure (i) showed no depression.

        α-Methoxy-α-methyl-2-naphthaleneacetic acid (IVa). To a solution of II (810 mg) in anhydrous dimethylformamide (4 ml) was added NaH (500 mg) under ice-cooling. The mixture was stirred for 30 min, then methyl iodide (4 ml) was added and the resulting solution was stirred for 45 min at room temperature. The reaction mixture was poured into ice-water and extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous Na_2SO_4, and evaporated. A crystalline product obtained was dissolved in 5 % methanolic KOH (20 ml) and heated at 50° for 1.5 h. The resulting solution was concentrated under reduced pressure, acidified with 5 % HCl, and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from ethyl acetate–hexane gave IVa (750 mg) as colourless plates [m.p. 52–53°; analysis: calculated for $C_{14}H_{14}O_3$: C, 73.02 %; H, 6.13 %; found: C, 72.72 %; H, 6.03 %; NMR (chloroform-d_3)δ: 1.96 (3H, s, –CH_3), 3.32 (3H, s, –OCH_3), 7.40–8.00 (7H, m, Ar–H), 9.55 (1H, s, –COOH)].

        (—)-α-Methoxy-α-methyl-2-naphthaleneacetic acid (IVb). To a solution of IVa (1 g) in ethanol (3 ml) was added (+)-α-methylbenzylamine (420 mg) in ethanol (3 ml). The mixture was heated on the steam bath to dissolve the salt that formed immediately, and allowed to stand at room temperature for 20 h. The precipitate was collected by filtration and fractionally crystallized from ethanol several times. The salt was decomposed with 5 % HCl and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization of the crude product from ether–hexane gave IVb (180 mg) as colourless plates [m.p. 67–68°; $[\alpha]_D^{17}$ —63.8° (c = 0.08, chloroform); analysis: calculated for $C_{14}H_{14}O_3$: C, 73.02 %; H, 6.13 %; found: C, 72.85 %; H, 6.09 %)].

        α-Hydroxy-α-methyl-1-naphthaleneacetic acid (VI). To a stirred solution of magnesium metal (200 mg) in anhydrous ether (8 ml) were added 1-bromonaphthalene (2 g) in anhydrous ether (2 ml) and a trace amount of methyl iodide. The mixture was

stirred at room temperature for 30 min under a stream of nitrogen gas. The resulting solution of 1-naphthylmagnesium bromide (V) was slowly added to a solution of pyruvic acid (450 mg) in ether (3 ml) and stirred at room temperature for 30 min. The reaction mixture was treated in the same manner as described for compound II. Recrystallization of the crude product from acetone–hexane gave VI (600 mg) as colourless plates (m.p. 110–112°; analysis: calculated for $C_{13}H_{12}O_3$: C, 72.21%; H, 5.59%; found: C, 72.15%; H, 5.62%).

α-Methoxy-α-methyl-1-naphthaleneacetic acid (VIIa). Compound VI (1 g) was treated in the same manner as described for compound IVa. Recrystallization of the crude product from acetone–hexane gave VIIa (950 mg) as colourless plates [m.p. 161–162°; analysis: calculated for $C_{14}H_{14}O_3$: C, 73.02%; H, 6.13%; found: C, 73.02%; H, 6.11%; NMR (chloroform-d_3)δ: 2.01 (3H, s, –CH₃), 3.07 (3H, s, –OCH₃), 7.30–8.50 (7H, m, Ar–H), 9.45 (1H, s, –COOH); UV $\lambda_{max}^{methanol}$ nm (log ε): 263 (3.36), 271 (3.53), 281 (3.57), 287 (3.46), 292 (3.45)].

(—)-α-Methoxy-α-methyl-1-naphthaleneacetic acid (VIIb). Compound VIIa (1.8 g) was resolved by twice fractionally crystallizing the (+)-α-methylbenzylamine salt from ethanol. The salt was decomposed with 5% HCl and the crude product was recrystallized from ether–hexane to give VIIb (830 mg) as colourless plates (m.p. 111–112°; $[\alpha]_D^{13}$ −106.3° ($c = 0.16$, chloroform); $[\alpha]_D^{13}$ −128.8° ($c = 0.10$, methanol); analysis: calculated for $C_{14}H_{14}O_3$: C, 73.02%; H, 6.13%; found: C, 73.27%; H, 6.12%).

Materials

Amino acids were kindly donated by Ajinomoto (Kawasaki, Japan) and their methyl esters were prepared by treatment with methanol and hydrogen chloride in the usual manner. All the reagents used were of analytical grade. Solvents were purified by distillation prior to use.

High-performance liquid chromatography

The apparatus used was a Waters Model ALC/GPC 202 R401 high-performance liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) equipped with a UV detector monitoring the absorbance at 280 nm. The test samples were applied to the chromatograph by a Waters Model U6K sample loop injector with an effective volume of 2 ml. The μPorasil (1 ft. $\times$ $\frac{1}{4}$ in. I.D.) and μBondapak C_{18} (1 ft. $\times$ $\frac{1}{4}$ in. I.D.) columns (Waters Assoc.) were used under ambient conditions.

Preparation of derivatives

To a solution of amino acid methyl esters (*ca.* 100 μg) in pyridine (0.2 ml) were added IVb or VIIb (*ca.* 2 mg) and N,N'-dicyclohexylcarbodiimide (*ca.* 2 mg). The mixture was allowed to stand at room temperature for 30 min, and then diluted with ethyl acetate (*ca.* 0.5 ml), successively washed with 5% HCl, 5% NaHCO₃ and water, and dried over anhydrous Na₂SO₄. A 5-μl aliquot was injected with a microsyringe into the chromatograph.

RESULTS AND DISCUSSION

The design of a promising derivatization reagent for liquid chromatographic

resolution of enantiomeric amines via diastereomers requires the incorporation of suitable structural features, *i.e.* chirality leading to efficient resolution, a function reactive toward the amino group, and a chromophore responding with the satisfactory sensitivity in the UV detector. These requirements prompted us to prepare new Mosher-type[19] naphthalene derivatives as chiral reagents.

Our efforts were initially directed to the synthesis of (—)-α-methoxy-α-methyl-2-naphthaleneacetic acid (IVb). The Grignard reaction of 2-naphthylglyoxalic acid (I) with methylmagnesium iodide provided α-hydroxy-α-methyl-2-naphthaleneacetic acid (II). This synthetic route, however, was somewhat tedious and, therefore, an alternative method was developed. The reaction of pyruvic acid with 2-naphthyl-magnesium bromide (III) afforded II in reasonable yield. Methylation followed by alkaline hydrolysis furnished α-methoxy-α-methyl-2-naphthaleneacetic acid (IVa). The optical resolution of IVa was accomplished by fractional crystallization of the (+)-α-methylbenzylamine salt.

CH3
|
CH3O—C—COOH
VIIa:(±)
b:(—)

OCH3
|
—C—COOH
|
CH3
IVa:(±)
b:(—)
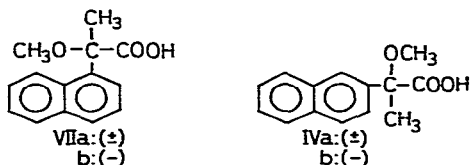
(—)-α-Methoxy-α-methyl-1-naphthaleneacetic acid (VIIb) was prepared similarly. The Grignard reaction of pyruvic acid with 1-naphthylmagnesium bromide (V) gave α-hydroxy-α-methyl-1-naphthaleneacetic acid (VI). Methylation and subsequent hydrolysis provided α-methoxy-α-methyl-1-naphthaleneacetic acid (VIIa). The racemate was readily resolved by fractionally crystallizing the (+)-α-methylbenzylamine salt to afford the desired compound (VIIb). The optical resolution was much easier with VIIa than with IVa.

Condensation of amino acid methyl ester with the reagent was effected by treatment with N,N′-dicyclohexylcarbodiimide in pyridine. The resulting amide was extracted with ethyl acetate and submitted to HPLC. The two chiral reagents were converted into the diastereomeric amides with L-proline methyl ester and chromatographed on a μPorasil column, and their optical purities proved to be both greater than 99.5%. In addition, the derivatization reagents were completely stable to racemization for prolonged reaction times.

The applicability of these chiral reagents to the liquid chromatographic resolution of the enantiomeric amines was investigated. The enantiomeric α-amino acid methyl esters in which the amine function leading to formation of the diastereomeric amides is attached to the centre of chirality, were taken as model compounds. Diastereomers were prepared from alanine, valine and proline methyl esters with the two derivatization reagents, and their chromatographic properties on a normal phase column were investigated (Table I). The k' and α values refer to the capacity ratio and separation factor for a pair of diastereomers, respectively. It is evident from the data that all the pairs of diastereomers derived from (—)-α-methoxy-α-methyl-1-naphthaleneacetic acid were resolved to a greater extent than those from the isomeric 2-naphthalene derivative. A marked difference in the chromatographic resolution between the positional isomers can be explained from a stereochemical point of view.

TABLE I

SEPARATION OF DIASTEREOMERIC N-(−)-α-METHOXÝ-α-METHYLNAPHTHALENE-ACETYLAMINO ACID METHYL ESTERS

Conditions: column, μPorasil (1 ft. × $^{1}/_{4}$ in. I.D.); mobile phase, cyclohexane–ethyl acetate (4:1), 0.7 ml/min; detection, 280 nm.

Amino acid	1-Naphthaleneacetyl			2-Naphthaleneacetyl		
	k' *		α	k'		α
	D	L		D	L	
Alanine	1.91	3.10	1.62	1.48	2.10	1.42
Valine	1.76	1.06	1.66	1.22	0.88	1.38
Proline	1 42	2.38	1.68	2.07	2.84	1.37

* $t_0 = 5.0$ min.

In the diastereomers formed from the 1-naphthaleneacetic acid derivative, interaction between the substituent at C-1 on the naphthalene nucleus and the hydrogen in the *peri* position may possibly occur. In contrast, this does not appear to be the case with the 2-naphthaleneacetic acid derivatives. On the basis of these results the more readily available (−)-α-methoxy-α-methyl-1-naphthaleneacetic acid was chosen as the more promising derivatization reagent.

The liquid chromatographic behaviour of N-(−)-α-methoxy-α-methyl-1-naphthaleneacetylamino acid methyl esters on reversed-phase and normal-phase columns was then examined more extensively. All the diastereomeric amides showed single, symmetrical peaks, indicating excellent chromatographic properties. On a μBondapak C_{18} column several pairs of diastereomers were partially resolved but

TABLE II

SEPARATION OF DIASTEREOMERIC N-(−)-α-METHOXY-α-METHYL-1-NAPHTHALENE-ACETYLAMINO ACID METHYL ESTERS ON A REVERSED-PHASE COLUMN

Conditions: column, μBondapak C_{18} (1 ft. × $^{1}/_{4}$ in. I.D.); mobile phase, methanol–water, (A) 2:1, (B) 3:2, 1 ml/min; detection, 280 nm.

Amino acid	k' *		α	R	Mobile phase
	D	L			
Alanine	1.83	1.83	1.00	0.00	A
Valine	3.06	2.66	1.15	0.92	A
Norvaline	3.33	2.95	1.13	0.75	A
Leucine	3.49	3.20	1.09	0.50	A
Norleuçine	4.80	3.87	1.24	1.00	A
Isoleucine	4.36	3.81	1.14	0.95	A
Proline	3.30	3.00	1.10	0.50	A
Phenylglycine	3.73	3.57	1.05	0.27	A
Phenylalanine	4.00	3.67	1.09	0.56	A
Threonine	2.80	2.64	1.06	0.23	B
Tyrosine	4.60	4.60	1.00.	0 00	B
Methionine	7.53	6.42	1.17	0.97	B
Tryptophan	11.21	11.00	1.02	0.15	B
Histidine	2.50	2.34	1.07	0.28	B

* $t_0 = 2.4$ min.

others were not separated (Table II). The resolution value, R, was calculated from the equation

$$R = 2 (t_{R_2} - t_{R_1})/(W_1 + W_2)$$

where t_{R_1} and t_{R_2} are retention times, and W_1 and W_2 are the bases of triangles derived from the peaks[20].

In sharp contrast, on a μPorasil column all the pairs of diastereomers, with two exceptions, were completely resolved (Table III). No marked difference in the separation between normal and branched-chain amino acids was detectable. Satisfactory resolution was also attained for aromatic, acidic and basic amino acids. Although no generalization can be made with regard to the elution order, it seems that the capacity ratios of L-amino acids are much more dependent on their structure than are those of the D-enantiomers. The chromatographic behaviour of aliphatic L-amino acids is influenced by the alkyl residue. Comparison of the k' values of alanine and serine with those of norvaline and threonine implies that the preferred conformation alters at a chain length of two or three carbon atoms.

The limits of detection and quantitation for the derivatized amino acid methyl ester by the use of a UV detector were found to be 10 ng and 50 ng, respectively. In addition, the sensitivity limit by fluorometric monitoring at λ_{max}^{ex} 305 nm and λ_{max}^{em}

TABLE III

SEPARATION OF DIASTEREOMERIC N-(−)-α-METHOXY-α-METHYL-1-NAPHTHALENE-ACETYLAMINO ACID METHYL ESTERS ON A NORMAL-PHASE COLUMN

Conditions: column, μPorasil (1 ft. × ¹/₄ in. I.D.); mobile phase, cyclohexane–ethyl acetate, (A) 4:1, (B) 2:1, (C) 1:1, (D) 2:3, 0.7 ml/min; detection 280 nm.

Amino acid	k' *		α	R	Mobile phase
	D	L			
Alanine	1.91	3.10	1.62	5.84	A
Valine	1.76	1.06	1.66	9.17	A
Norvaline	2.17	1.22	1.78	5.90	A
Leucine	1.83	0.78	2.35	6.14	A
Norleucine	1.92	0.96	2.00	6.00	A
Isoleucine	1.53	0.88	1.74	4.26	A
Proline	1.42	2.38	1.68	2.48	A
Phenylglycine	2.56	1.32	1.94	6.22	A
Phenylalanine	2.41	1.52	1.59	4.35	A
Serine	2.52	7.32	2.90	8.63	C
Threonine	1.20	1.85	1.55	2.98	D
Tyrosine	2.02	1.74	1.16	1.02	B
DOPA	2.32	2.04	1.14	0.67	C
Cysteine	2.08	0.98	2.12	2.80	C
Methionine	1.60	1.24	1.29	1.61	B
Aspartic acid	5.61	7.00	1.25	2.52	A
Glutamic acid	6.46	6.81	1.05	0.62	A
Ornithine	2.00	3.52	1.76	3.33	D
Lysine	1.60	2.20	1.38	1.97	D
Histidine	1.36	1.96	1.45	1.47	D

* $t_0 = 5.0$ min.

340 nm was one tenth less than that by UV absorption monitoring. Introduction of an electron-donating substituent, *e.g.* the dimethylamino group, into the aromatic nucleus may possibly improve the sensitivity for fluorometric monitoring.

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