

Enantioseparation of chiral amino acids as the *N(O,S)*-ethoxycarbonylated diastereomeric esters by achiral dual-capillary column gas chromatography

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The enantioseparation of 30 racemic amino acids in a single analysis is described for the determination of their absolute configurations. Two-phase extractive ethoxycarbonyl (EOC) reaction with ethyl chloroformate present in the dichloromethane phase was performed to recover amino acids from alkaline aqueous solutions. The resulting *N(O,S)*-EOC amino acids extracted into an organic solvent after acidification were reacted with a chiral alcohol such as (*S*)-(+)-3-methylbutan-2-ol, (*S*)-(+)-butan-2-ol and (*S*)-(+)-octan-2-ol for gas chromatographic analysis on achiral dual-capillary DB-5 and DB-17 columns of different polarities. Among the chiral reagents examined, (*S*)-(+)-3-methylbutan-2-ol provided the best diastereomeric structures in resolving all the racemic amino acids into their enantiomeric pairs with high resolution factors (1.2–8.0). Moreover, the temperature-programmed retention index (*I*) values measured on the two columns were characteristic of each enantiomer. Hence simple *I* matching with the reference values was useful in cross-checking for chemical identification and also chiral discrimination. When the present method was applied to a fermented dairy product (Yakult), D-alanine, D-aspartic acid, D-glutamic acid and D-proline were positively detected along with their respective L-forms in addition to glycine.

Aim of investigation

With the discovery of D-amino acids in living organisms and fermented products and with the growing concern about their biological activity, the accurate configurational analysis of amino acids has become an important task in various fields.^{1–8} The simultaneous detection and identification of multiple amino acids in a single run are thus frequently encountered when screening unknown samples for D-amino acids.^{5–12}

Among the various techniques developed for this purpose based on high-performance liquid chromatography (HPLC) and gas chromatography (GC), indirect HPLC methods after conversion of amino acids into fluorescent diastereomeric derivatives have been widely used to achieve higher sensitivity.^{5,6} When employing GC combined with mass spectrometry (MS) to achieve higher enantiomeric resolution in a single run, both direct and indirect approaches require amino acids to be pre-converted into volatile derivatives.^{3,8,9,13–19} In consideration of this prerequisite, indirect enantioseparation as volatile diastereomeric derivatives using conventional achiral stationary phases with high thermal stability and long-term durability appears to be preferred over the direct approach.^{3,13–16} Most tailor-made chiral capillary columns for the direct methods are known to be thermally unstable, thus requiring conversion of amino acids into much more volatile derivatives.^{17,18}

In earlier studies, esterification of carboxyl groups with a homochiral alcohol with subsequent *N*-acylation^{14,15} was frequently used for the diastereomeric reaction. The converse approach, *N*-acylation with a chiral acylating agent after esterification, was rarely used.¹³ These old methods require time-consuming, laborious steps for complete moisture removal from aqueous amino acid samples. As a different approach, alkoxycarbonyl (AOC) reaction of amino acids with menthyl

chloroformate has been performed directly in aqueous alcohol media containing pyridine for conversion into their corresponding diastereomeric *N*-menthoxy carbonyl methyl or ethyl ester derivatives in a single treatment.¹⁶ This simultaneous AOC reaction and esterification in one-step has been extensively studied for the achiral separation of amino acids and its usefulness was well documented in a recent review.²⁰ However, this rapid one-step method still has limitations for direct application to aqueous amino acid extracts without prior reduction to sub-milliliter volumes. In our preliminary studies, the menthoxy carbonyl group was found to be too bulky for multifunctional amino acids. Hence the AOC reaction with smaller alkyl chloroformates in aqueous alkaline solutions to convert *N(O,S)*-AOC amino acids into solvent-extractable free carboxylic acid forms, followed by esterification or silylation, appears to be more suitable for a wider range of amino acids including non-protein amino acids. Previously, we reported that simultaneous achiral analysis of 51 amino acids in a single run was successfully achieved as their *N(O,S)*-isobutoxy carbonyl *tert*-butyldimethylsilyl derivatives.^{21,22} This direct AOC procedure in aqueous solutions, however, required large amounts of alkyl chloroformates because of their instability in alkaline solution. This problem was overcome by employing extractive two-phase AOC reaction in our more recent work.^{23,24}

The chirality of each separated amino acid enantiomer is mainly determined by co-chromatography with the enantiomerically pure D- or L-amino acid standards, necessitating at least a second GC run with real samples. Accordingly, it is very desirable to develop an efficient method for simultaneous peak identification and determination of chiral configuration. In our previous work,^{25,26} achiral dual-capillary columns of different polarities were found to be useful in configurational analysis of chiral acids, allowing accurate identification and chiral discrim-

ination of each enantiomer by retention index (*I*) matching with reference values. Therefore, the tedious problems with the conventional co-chromatographic procedure were solved and the overall analysis time was shortened considerably.

In continuation of our amino acid screening work,^{21,22} this study was undertaken to extend the previous GC *I* matching system^{25,26} to the accurate configurational determination of the screened amino acids as their diastereomeric derivatives. In this study, the two-phase extractive ethoxycarbonyl (EOC) reaction was employed for the recovery of 29 racemic amino acids and *S*-methyl-D-cysteine from aqueous samples followed by diastereomeric esterification. (*S*)-(+)-3-Methylbutan-2-ol, (*S*)-(+)-butan-2-ol and (*S*)-(+)-octan-2-ol were examined for their ability to form optimal diastereomeric structures that provide good enantioseparation of each racemic amino acid. The resulting *N*(*O,S*)-EOC esters were subjected to GC analysis on thermally stable achiral dual-capillary columns of different polarities in an attempt to achieve simultaneous chemical identification and accurate chiral discrimination based on GC *I* matching.

2. Experimental

2.1 Materials

The following 29 racemic amino acid standards and 26 enantiomerically pure standards were obtained from various vendors such as Sigma-Aldrich (St. Louis, MO, USA): alanine, valine, leucine, isoleucine, allo-isoleucine, proline, aspartic acid, phenylalanine, lysine, methionine, cysteine, glutamic acid, tryptophan, tyrosine, α -aminobutyric acid, β -aminoisobutyric acid, norvaline, pipercolinic acid, 2-phenylglycine, selenomethionine, selenoethionine, homophenylalanine, ornithine, norleucine, ethionine, 2,3-diaminopropionic acid, homocysteine,

α -aminoadipic acid, α -aminopimelic acid, L-alanine, L-valine, L-leucine, L-isoleucine, L-allo-isoleucine, L-proline, L-aspartic acid, L-phenylalanine, L-lysine, L-methionine, L-cysteine, L-glutamic acid, L-tryptophan, L-tyrosine, L- α -aminobutyric acid, L-norvaline, L-pipercolinic acid, *S*-methyl-L-cysteine, L-2-phenylglycine, L-selenomethionine, L-homophenylalanine, L-ornithine, L-norleucine, L-ethionine, L-2,3-diaminopropionic acid and L- α -aminoadipic acid. (*S*)-(+)-3-Methylbutan-2-ol, (*S*)-(+)-butan-2-ol, (*S*)-(+)-octan-2-ol, acetyl chloride and ethyl chloroformate (ECF) were also purchased from Sigma-Aldrich and n-alkane standards (C₁₀–C₃₂, even numbers only) from Polyscience (Niles, IL, USA). Diethyl ether, toluene, dichloromethane and isooctane of spectroanalyzed grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical-reagent grade and used as received.

2.2. Amino acid and hydrocarbon standard solutions

Each standard solution of amino acids was made up at 10 μg μl^{-1} in 0.1 M HCl as their free acid forms. Hydrocarbon solution containing n-alkanes (C₁₀–C₃₂, even numbers only), each at 1.0 μg μl^{-1} in isooctane, was used as the external standard solution for *I* measurements.

2.3 *N*(*O,S*)-Ethoxycarbonylation with subsequent diastereomeric esterification

An aliquot (1 ml) of sodium phosphate buffer (pH 8) containing amino acids (each at 10 μg) was added to dichloromethane (1 ml) containing ethyl chloroformate (20 μl). After the mixture had been vortex mixed (10 min) at room temperature, its pH was adjusted to 11–12 with 5M NaOH and then it was vortex mixed (10 min) at room temperature. The reaction mixture was

Table 1 Resolution factors of amino acid enantiomers as their three diastereomeric *N*(*O,S*)-ethoxycarbonylated esters^a

No.	Chiral amino acid	3-Methyl-2-butyl ester		2-Butyl ester		2-Octyl ester	
		DB-5	DB-17	DB-5	DB-17	DB-5	DB-17
1	Alanine	3.3	5.8	2.9	3.3	4.5	4.2
2	α -Aminobutyric acid	4.2	4.8	2.7	2.4	4.2	3.9
3	β -Aminoisobutyric acid	3.2	2.4	2.2	2.1	3.5	3.8
4	Valine	4.4	5.0	2.5	3.2	4.6	4.6
5	Norvaline	5.4	4.2	2.0	2.1	3.9	3.9
6	Leucine	4.2	4.4	2.5	4.2	3.9	3.5
7	allo-Isoleucine	3.8	4.0	2.7	3.6	4.1	3.6
8	Isoleucine	8.0	6.0	4.9	6.7	7.2	6.4
9	Norleucine	3.9	4.4	2.5	2.6	3.4	3.3
10	Proline	1.7	1.7	1.1	0.9	2.2	2.2
11	Pipercolic acid	1.5	1.5	0.8	0.9	2.7	2.6
12	<i>S</i> -Methylcysteine	2.9	3.2	1.4	1.6	2.1	2.0
13	Methionine	2.8	3.3	1.5	1.8	2.3	1.7
14	2-Phenylglycine	2.3	2.5	0.9	1.1	NS ^b	NS
15	Ethionine	2.6	3.2	1.5	1.7	1.6	1.4
16	Selenomethionine	2.8	2.7	1.5	1.8	1.9	1.6
17	Aspartic acid	1.2	1.8	0.9	1.3	0.8	1.0
18	2,3-Diaminopropionic acid	1.7	2.2	<0.75	1.0	NS	NS
19	Phenylalanine	1.9	3.0	1.2	1.7	2.2	1.9
20	Selenoethionine	2.7	2.8	1.3	1.7	1.4	1.2
21	Cysteine	2.0	2.4	1.1	1.5	1.0	0.9
22	Glutamic acid	2.7	2.4	1.5	1.5	0.8	0.9
23	Homophenylalanine	2.7	3.1	1.4	1.5	1.4	1.0
24	Homocysteine	2.2	2.4	1.0	1.4	<0.75	NS
25	α -Aminoadipic acid	2.4	1.8	1.3	1.4	NS	NS
26	Ornithine	2.4	2.8	1.1	1.3	NS	NS
27	α -Aminopimelic acid	2.4	2.7	1.1	1.3	NS	NS
28	Lysine	2.2	2.6	1.0	1.2	NS	NS
29	Tyrosine	1.7	2.0	<0.75	1.1	NS	NS
30	Tryptophan	1.9	2.4	0.9	1.1	NS	NS

^a Resolution factors are the ratios of separation between two peaks to their average peak width at the baseline. ^b Not separated.

adjusted to pH 1–2 with concentrated sulfuric acid and saturated with sodium chloride, followed by extraction with diethyl ether (2 ml × 3). The ethereal extract was dried over magnesium sulfate and evaporated to dryness (under a gentle stream of nitrogen). To the dried residue were added toluene (20 µl), (*S*)-(+)-3-methylbutan-2-ol (30 µl) and acetyl chloride (1 µl). The mixture was then heated at 100 °C for 1.5 h. The excess reagents were removed (under a stream of nitrogen) and the residue was reconstituted in toluene (30 µl) for direct analysis by GC and GC-MS. In place of (*S*)-(+)-3-methylbutan-2-ol, the esterification reaction with (*S*)-(+)-butan-2-ol or (*S*)-(+)-octan-2-ol was performed under identical conditions.

2.4 Sample preparation

An aliquot (1.0 ml) of locally purchased Yakult samples was centrifuged for 10 min at 2500 rpm. The upper layer (500 µl) was subjected to *N*(*O,S*)-ethoxycarbonylation with subsequent diastereomeric esterification with (*S*)-(+)-3-methylbutan-2-ol as described above.

2.5 Gas chromatography and gas chromatography-mass spectrometry

GC analyses were performed with a Hewlett-Packard (HP) Model 5890A Series II gas chromatograph, equipped with a split/splitless capillary inlet system and two flame ionization detectors and interfaced to an HP 3365A GC Chemstation (Hewlett-Packard, Avondale, PA, USA). The injector and detector temperatures were 260 and 280 °C, respectively.

Samples (*ca.* 0.5 µl) were injected in the splitless mode with a purge delay time of 0.7 min. Retention index (*I*) measurements were carried out using a dual-capillary column system with DB-5 (SE-54 bonded phase) and DB-17 (OV-17 bonded phase) fused-silica capillary columns (J & W Scientific, Rancho Cordova, CA, USA) (30 m × 0.25 mm id, 0.25 µm film thickness). The two columns were connected to deactivated fused-silica tubing (1 m × 0.25 mm id) as retention gap *via* a Y-splitter. The inlet pressure of helium as the carrier gas was set to 137.5 kPa. For the (*S*)-(+)-3-methyl-2-butyl esters, the oven temperature was held at 60 °C for 1 min, then programmed at 30 °C min⁻¹ to 120 °C (held for 2 min) and subsequently programmed to 280 °C at 3 °C min⁻¹. In the case of the (*S*)-(+)-2-butyl esters, the oven temperature was initially 60 °C for 1 min, then programmed at 30 °C min⁻¹ to 100 °C (held for 2 min) and finally to 280 °C at 3 °C min⁻¹. For the (*S*)-(+)-2-octyl esters, the oven temperature was programmed from 60 °C (held for 1 min) to 180 °C (held for 2 min) at 30 °C min⁻¹ and then to 280 °C at 3 °C min⁻¹. A standard solution of n-alkanes (C₁₀–C₃₂, even numbers only) in isooctane was injected as the external references and temperature-programmed *I* values were computed *via* a built-in retention index program by linear interpolation between the retention times of adjacent alkane standards. For the peak identification and chirality determination by computer *I* matching, a database of reference *I* library using *I* sets of 60 chiral amino acids measured on the dual columns was built into the GC computer system. To obtain mass spectra, an HP 5890A Series II gas chromatograph, interfaced to an HP 5970B mass spectrometer (70 eV, electron ionization mode), which was on-line to an HP 59940A MS Chemstation, was used. Samples were injected into an Ultra-2 (SE-54 bonded phase) capillary column (25 m × 0.20 mm id, 0.11 µm film thickness) in the split injection mode (10:1) at 260 °C and the

Table 2 Retention index (*I*) values of 30 amino acids as diastereomeric *N*(*O,S*)-EOC (*S*)-(+)-3-methyl-2-butyl esters

No.	Chiral amino acid	GC <i>I</i> data set ^a		No.	Chiral amino acid	GC <i>I</i> data set ^a	
		DB-5	DB-17			DB-5	DB-17
1	D-Alanine	1458.3	1652.0	16	D-Selenomethionine	1970.6	2278.9
	L-Alanine	1473.3	1671.0		L-Selenomethionine	1980.2	2291.2
2	D-α-Aminobutyric acid	1532.7	1729.5	17	D-Aspartic acid	2010.5	2264.8
	L-α-Aminobutyric acid	1545.5	1744.8		L-Aspartic acid	2016.4	2271.7
3	β-Aminoisobutyric acid	1571.0	1786.9	18	D-2,3-Diaminopropionic acid	2022.2	2352.7
	β-Aminoisobutyric acid	1579.1	1796.7		L-2,3-Diaminopropionic acid	2029.5	2362.3
4	D-Valine	1573.0	1765.4	19	D-Phenylalanine	2026.9	2343.5
	L-Valine	1584.8	1780.6		L-Phenylalanine	2034.4	2355.0
5	D-Norvaline	1605.5	1801.5	20	Selenoethionine	2039.4	2338.0
	L-Norvaline	1617.4	1815.6		Selenoethionine	2048.8	2349.1
6	D-Leucine	1638.1	1824.7	21	D-Cysteine	2066.1	2394.0
	L-Leucine	1649.8	1839.3		L-Cysteine	2073.9	2405.1
7	D-allo-Isoleucine	1647.4	1839.3	22	D-Glutamic acid	2138.5	2394.0
	L-allo-Isoleucine	1659.4	1853.7		L-Glutamic acid	2148.7	2405.1
8	D-Isoleucine	1647.4	1839.3	23	D-Homophenylalanine	2160.9	2478.6
	L-Isoleucine	1671.1	1868.3		L-Homophenylalanine	2170.9	2491.9
9	D-Norleucine	1692.9	1886.5	24	Homocysteine	2188.4	2528.9
	L-Norleucine	1704.7	1901.0		Homocysteine	2197.3	2540.9
10	D-Proline	1692.7	1973.9	25	D-α-Amino adipic acid	2250.7	2519.6
	L-Proline	1697.5	1979.4		L-α-Amino adipic acid	2259.6	2528.9
11	D-Pipecolic acid	1750.6	2015.6	26	D-Ornithine	2271.8	2646.8
	L-Pipecolic acid	1755.1	2021.1		L-Ornithine	2281.7	2659.4
12	<i>S</i> -Methyl-D-Cysteine ^b	1789.1	2072.0	27	α-Aminopimelic acid	2349.1	2627.6
	<i>S</i> -Methyl-L-cysteine	1797.5	2084.2		α-Aminopimelic acid	2357.5	2638.9
13	D-Methionine	1904.7	2199.5	28	D-Lysine	2373.9	2760.4
	L-Methionine	1914.2	2212.3		L-Lysine	2383.3	2772.3
14	D-2-Phenylglycine	1932.1	2252.5	29	D-Tyrosine	2549.9	2974.6
	L-2-Phenylglycine	1936.8	2261.9		L-Tyrosine	2557.7	2985.5
15	D-Ethionine	1971.7	2258.0	30	D-Tryptophan	2656.0	3234.9
	L-Ethionine	1980.4	2269.6		L-Tryptophan	2664.8	3247.6

^a Retention index (*I*) measured on DB-5 and DB-17 (30 m × 0.25 mm id, 0.25 µm film thickness) dual capillary columns. ^b The elution order and the *I* value were determined in the enantiomeric form of *N*(*O,S*)-EOC *S*-methyl-L-cysteine (*R*)-(-)-3-methyl-2-butyl ester prepared by reacting enantiomerically pure *S*-methyl-L-cysteine with racemic 3-methylbutan-2-ol.

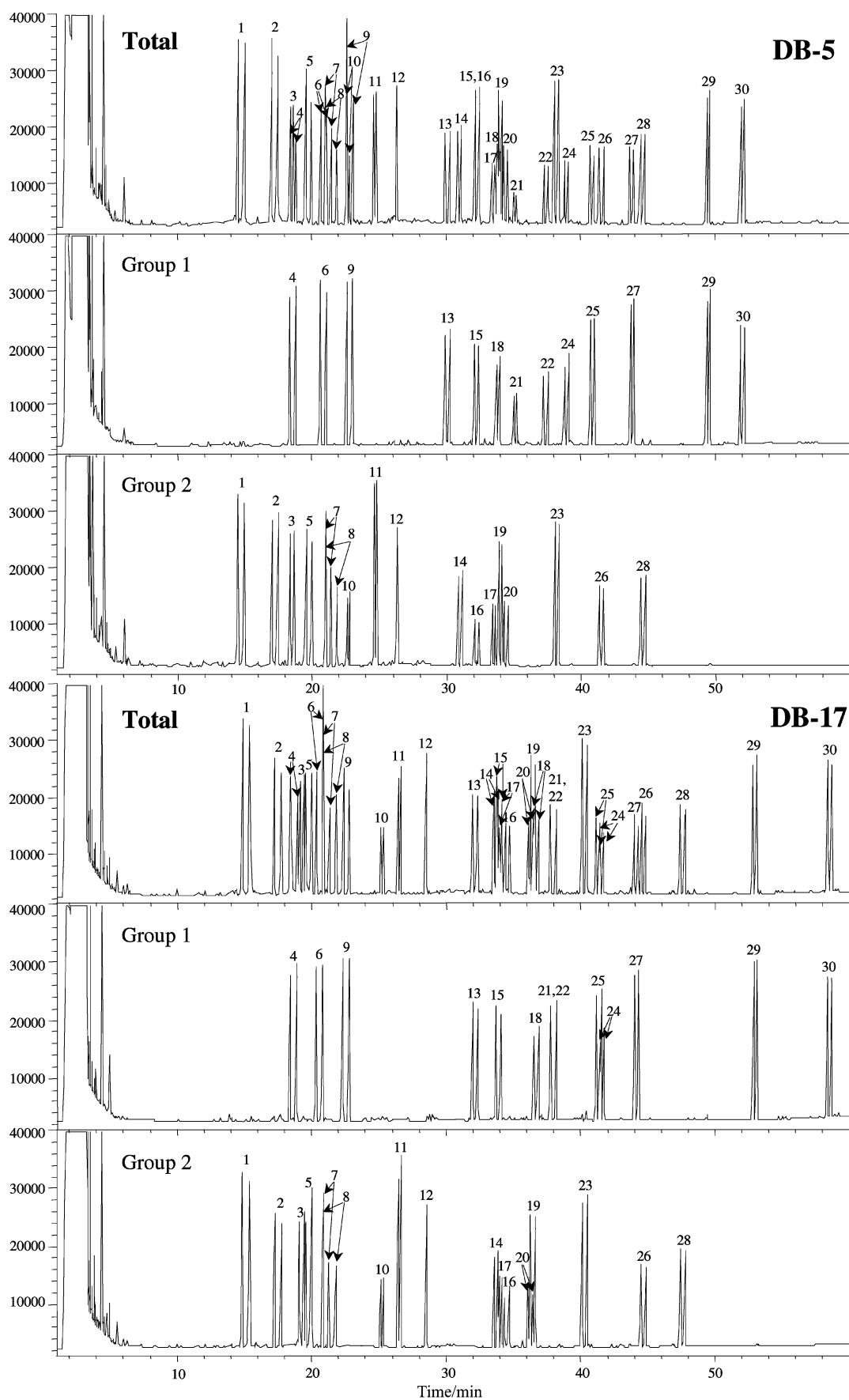


Fig. 1 Dual chromatograms of enantiomeric pairs of 30 amino acids as their diastereomeric *N(O,S)*-EOC (*S*)-(+)-3-methyl-2-butyl esters separated on DB-5 and DB-17 (both 30 m × 0.25 mm id, 0.25 μm film thickness) dual-capillary column system. The oven temperature was held at 60 °C for 1 min and programmed to 120 °C at a rate of 30 °C min⁻¹, then to 280 °C at a rate of 3 °C min⁻¹. Samples (ca. 0.5 μl) were injected in the splitless mode with a purge delay time of 0.7 min. Peaks: 1 = alanine; 2 = α-aminobutyric acid; 3 = β-aminoisobutyric acid; 4 = valine; 5 = norvaline; 6 = leucine; 7 = allo-isoleucine; 8 = isoleucine; 9 = norleucine; 10 = proline; 11 = pipercolinic acid; 12 = *S*-methylcysteine; 13 = methionine; 14 = 2-phenylglycine; 15 = ethionine; 16 = selenomethionine; 17 = aspartic acid; 18 = 2,3-diaminopropionic acid; 19 = phenylalanine; 20 = selenoethionine; 21 = cysteine; 22 = glutamic acid; 23 = homophenylalanine; 24 = homocysteine; 25 = α-aminoadipic acid; 26 = ornithine; 27 = α-aminopimelic acid; 28 = lysine; 29 = tyrosine; 30 = tryptophan.

oven temperature was initially 120 °C for 2 min and then raised to 280 °C at 3 °C min⁻¹. The interface and ion source temperatures were 280 and *ca.* 250 °C, respectively. The mass range scanned was 50–650 u at a rate of 0.99 scan s⁻¹.

3 Results and discussion

3.1 *N(O,S)*-Ethoxycarbonylation with subsequent diastereomeric esterification

The present extractive two-phase *N(O,S)*-EOC reaction combined with a pH shift required a minimal amount (20 µl) of ethyl chloroformate to recover most of the amino acids efficiently from aqueous solution into the organic phase. However, of protein amino acids, glutamine, asparagine and histidine were more poorly recovered from aqueous solutions than with our previous *N(O,S)*-isobOC reaction with isobutyl chloroformate.^{21,22} As reported elsewhere,^{21,22,27} arginine was converted into ornithine during the EOC reaction.

Upon the diastereomeric esterification of the recovered *N(O,S)*-EOC amino acids for direct subsequent GC analysis on the dual-columns, a single derivative was obtained from each of the 60 amino acid enantiomers tested. Their chemical structures were confirmed by GC-MS. Racemization during the reactions was not observed.

Among the three chiral alcohols examined, (*S*)-(+)-3-methylbutan-2-ol yielded esters with the best diastereomeric structures. When the racemic amino acids were individually analyzed, complete enantioseparation for all the amino acids was achieved on both non-polar DB-5 and intermediately polar DB-17 columns with resolution factors in the range 1.2–8.0 (Table 1). When butan-2-ol was used, the respective resolution factors were, however, considerably reduced except for isoleucine (Table 1). With octan-2-ol, resolution factors higher than 1.4 were achieved only for 18 amino acid pairs while three pairs were partially resolved and nine pairs were unresolved. The present findings suggest that the alkyl group adjacent to the chiral center of a chiral reagent requires to be branched and thus rigid such as the isopropyl group of 3-methylbutan-2-ol to

achieve good enantioseparation (resolution factors ≥ 1.4) of structurally diverse amino acids.

3.2 Chiral profiling and screening for amino acids as *N(O,S)*-EOC (*S*)-(+)-3-methyl-2-butyl esters

For the extension of our amino acid screening method based on simple *I* matching using a dual-capillary column system^{21,22} to the chiral discrimination of amino acids, the identical achiral column system was employed to resolve enantiomeric pairs of 30 amino acids as their diastereomeric (*S*)-(+)-3-methyl-2-butyl esters. The elution orders and *I* values of 26 enantiomeric pairs were determined using authentic enantiopure amino acid standards of established absolute configuration. For *S*-methyl-D-cysteine those values were determined in the form of *N(O,S)*-EOC *S*-methyl-L-cysteine (*R*)-(-)-3-methyl-2-butyl ester prepared by reacting enantiomerically pure *S*-methyl-L-cysteine with racemic 3-methylbutan-2-ol.

In all cases D-enantiomers emerged before the corresponding L-enantiomers on both columns (Table 2). These results were in good agreement with the previous findings with *N*-pentafluoropropionyl (*S*)-(+)-3-methyl-2-butyl ester derivatives on a non-polar SE-30 column.¹⁵ In a recent study, the same results were obtained as the *N*-(+)-menthoxy carbonyl methyl or ethyl esters on both polar DB-210 and intermediately polar OV-1701 columns, except for 2-phenylglycine.¹⁶

Under the present achiral GC conditions, baseline resolutions for most of the amino acids were simultaneously achieved on the dual-capillary columns in a single run within 1 h (Fig. 1). No resolutions among L-leucine (peak 6), D-allo-isoleucine (peak 7) and D-isoleucine (peak 8) were achieved on either column. Resolutions between D-norleucine (peak 9), D-proline (peak 10), D-ethionine (peak 15) and D-selenomethionine (peak 16), L-ethionine (peak 15) and L-selenomethionine (peak 16) and L-2,3-diaminopropionic acid (peak 18) and D-phenylalanine (peak 19) were achieved on DB-17 but were not feasible on DB-5. In contrast, cysteine (peak 21) and glutamic acid (peak 22) were resolved on DB-5 but not on DB-17. The first enantiomer of homocysteine (peak 24) co-eluted with L- α -amino adipic acid (peak 25) on DB-17. It was noted that the elution orders of most amino acids in racemic form on the two columns were the same,

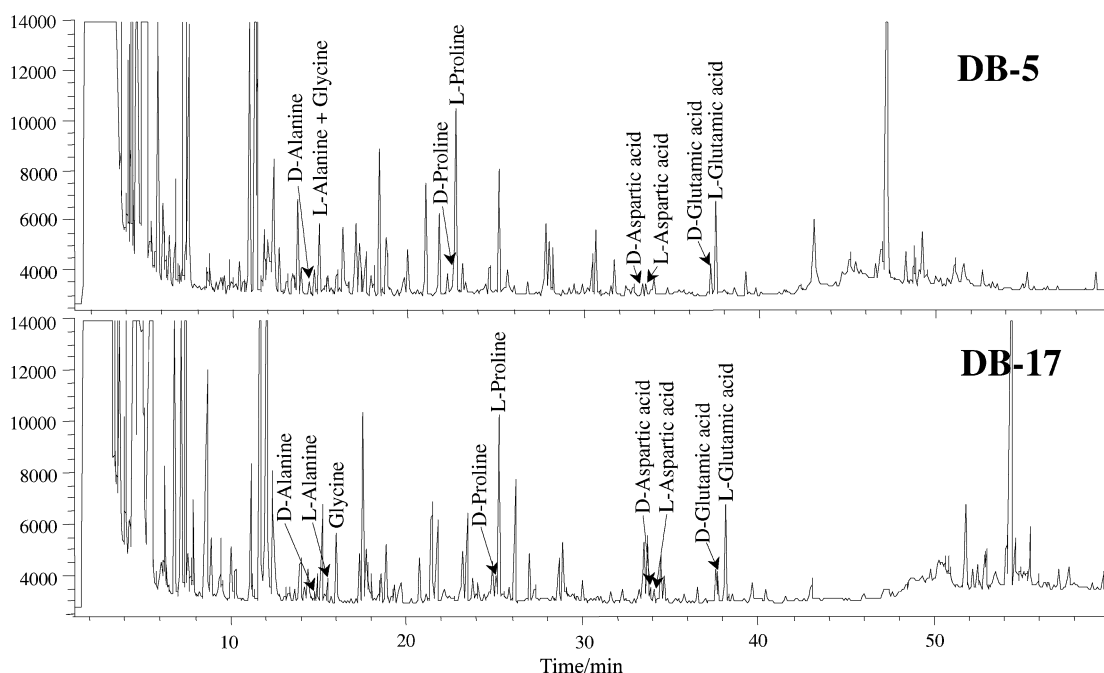


Fig. 2 Dual chromatographic profiles of amino acids from Yakult sample as diastereomeric *N(O,S)*-EOC (*S*)-(+)-3-methyl-2-butyl ester derivatives separated on DB-5 and DB-17 (both 30 m \times 0.25 mm id, 0.25 µm film thickness) dual-capillary column system. GC conditions as in Fig. 1.

with the exception of four sets: β -aminoisobutyric acid (peak 3) and valine (peak 4); 2,3-diaminopropionic acid (peak 18) and selenomethionine (peak 20); homocysteine (peak 24) and α -aminoadipic acid (peak 25); and ornithine (peak 26) and α -aminopimelic acid (peak 27).

As manifested in Table 2, the temperature-programmed *I* values measured simultaneously on the dual-capillary columns were characteristic of each resolved enantiomer. Therefore, the present method based on simple *I* matching with the reference values was useful in cross-checking for the chemical identification of amino acids and their chiral discrimination. In preliminary experiments on the application of the present method to various fermented food products, D-alanine, D-aspartic acid, D-glutamic acid and D-proline were positively detected in Yakult samples along with their respective L-forms in addition to glycine (Fig. 2). These results were comparable to previous findings in the literature,⁷ except for D-proline, which has not been detected in fermented dairy products.^{7,8}

At present, our chiral amino acid *I* library contains enantiomeric pairs of 30 amino acids as their *N*(*O,S*)-EOC (*S*)-(+)-3-methyl-2-butyl esters, and will continue to be expanded to include other non-protein amino acids for their screening and configurational analysis from a wide range of natural samples. Also, an extension of the present chiral discrimination method to more efficient diastereomeric derivatization procedures to include serine and threonine is under way to be used as a complementary confirmation tool in configurational analysis.

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