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ISOCYANATES AS REAGENTS FOR ENANTIOMER SEPARATION: APPLICATION TO AMINO ACIDS, N-METHYLAMINO ACIDS AND 3-HYDROXY ACIDS

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SUMMARY

The formation of N-alkylureido derivatives of amino acid esters increases the selectivity of a chiral stationary phase towards the enantiomers, resulting in complete separation, even for the proline and isovaline enantiomers. In the reaction of isocyanates with N-methylamino acids, N-alkylureido/N-alkylamide derivatives are formed in one step. In a similar way, 3-hydroxy acids are converted into N-alkylcarbamate/N-alkylamide derivatives. By this procedure, the enantiomers of N-methylamino and 3-hydroxy acids were separated on chiral stationary phases for the first time. The order of elution of the enantiomers of 3-hydroxy acids was determined with optically active reference compounds, obtained by stereoselective reduction of 3-keto acid esters with yeast.

INTRODUCTION

Enantiomer separation by capillary gas chromatography (GC) has been greatly stimulated by the introduction of thermostable chiral stationary phases, *e.g.*, Chirasil-Val by Frank *et al.*[1,2] and XE-60–L-valine-(*S*)- or (*R*)-α-phenylethylamide by our group[3,4]. The enhanced temperature stability of these stationary phases also allows the use of less volatile and more polar derivatives of enantiomers. In this way, substituents capable of selective molecular interactions with the polar groups of the stationary phase can be introduced. We have demonstrated that carbamate and ureido derivatives are superior for the enantiomer separation of chiral alcohols[5], α-hydroxy acids and amines[6]. Moreover, preliminary investigations have shown that the separation of N-methylamino and β-hydroxy acids may be possible after preparing ureido or carbamate derivatives and by simultaneously converting the carboxylic groups to amides[7,8].

In this work, we discuss the results we have obtained with isocyanates as reagents for enantiomer separations of amino acids, N-methylamino acids and 3-hydroxy acids.

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EXPERIMENTAL

Materials

Isocyanates were kindly supplied by Bayer (Leverkusen, F.R.G.). N-Methylamino acids were prepared according to the procedure of McDermott and Benoiton[9]; racemic 3-hydroxy acid ethyl esters were synthesized in a Reformatskii reaction[10] and hydrolysed to the free acids in 6 *N* hydrochloric acid at 100°C for 18 h. Optically active 3-hydroxy acids were obained by enzymatic stereoselective reduction of β-keto acid esters as described by Mori and Tanida[11].

Formation of derivatives

Amounts of 100 μg of racemic amino acids and pure enantiomers were esterified in 1 ml of 1.5 *N* hydrochloric acid–isopropanol at 100°C in a screw-capped vial. After removal of excess of reagent by a stream of nitrogen, 100 μl of dichloromethane, 5 μl of dry pyridine and 100 μl of *tert.*-butyl isocyanate were added to the sample, which was then kept at room temperature for 1 h. Heating of the reaction mixture for 15 min at 100°C in some instances improved the yield of derivatives. Hydroxyamino acids were silylated at room temperature with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). N-Methylamino acids were heated in a screw-capped vial with 150 μl of dichloromethane and 150 μl of either isopropyl- or *tert.*-butylisocyanate for 15 min at 100°C. 3-Hydroxy acids were derivatized in the same way, but with heating for 1 h. The exces of reagents was removed in a stream of nitrogen and the sample was dissolved in dichloromethane for GC.

Gas chromatography

The chiral stationary phases XE-60–L-valine-(*S*)- and -(*R*)-α-phenylethylamide were prepared as described earlier[3,4]. Pyrex glass capillary columns with a Silanox interlayer[12] or fused-silica capillary columns (Chrompack, Middelburg, The Netherlands), coated with the above-mentioned chiral phases, were used in Carlo Erba Model 2101 gas chromatographs with hydrogen as the carrier gas. The derivatives were investigated by GC–mass spectrometry (GC–MS) with a Hewlett-Packard HP 5985 A instrument.

RESULTS AND DISCUSSION

Amino acids

Enantiomer separation of N-trifluoroacetylated amino acid esters was the first successful application of enantioselective GC[13] and has been further developed to the present high standard by Frank *et al.*[1,2,14]. Nevertheless, this method has its limitations. It is difficult to separate the enantiomers of proline, of pipecolic acid (homoproline) and of α-alkylated amino acids, and it is not possible to separate N-methylamino acids.

In accordance with the reaction of chiral amines[6], α-amino acid esters are converted to N-ureido derivatives with isocyanates at room temperature or by brief heating in the presence of pyridine. When isopropyl isocyanate is used, a mixture of the mono- and bis-N-isopropylureido derivatives is obtained, as demonstrated by GC–MS, whereas *tert.*-butyl isocyanate forms exclusively the mono-derivatives. This

TABLE I

SEPARATION FACTORS (α) AND OPERATING TEMPERATURES FOR SEPARATION OF N-*tert.*-BUTYL-UREIDO-D,L-AMINO ACID ISOPROPYL ESTERS ON A 25-m GLASS CAPILLARY COLUMN WITH XE-60-L-VALINE-(*S*)-α-PHENYLETHYLAMIDE (IN ORDER OF EMERGENCE)

Amino acid	α	*Column temperature (°C)*
Iva*	1.02	170
Pro	1.03	180
Pyroglu	1.02	160
Pipecolic acid	1.03	160
Ala	1.09	180
Abu	1.08	180
Val	1.08	180
Nva	1.09	180
a-Ile	1.09	180
Thr (OTMS)**	1.09	180
a-Thr (OTMS)**	1.05	180
Leu	1.09	180
Ile	1.08	180
Ser (OTMS)**	1.11	180
Nle	1.09	180
Asp	1.08	180
Met	1.07	200
Phg***	1.02	200
Glu	1.07	200
Phe	1.07	200

* Iva = isovaline.
** Side-chain hydroxy group was silylated with MSTFA.
*** Phg = phenylglycine.

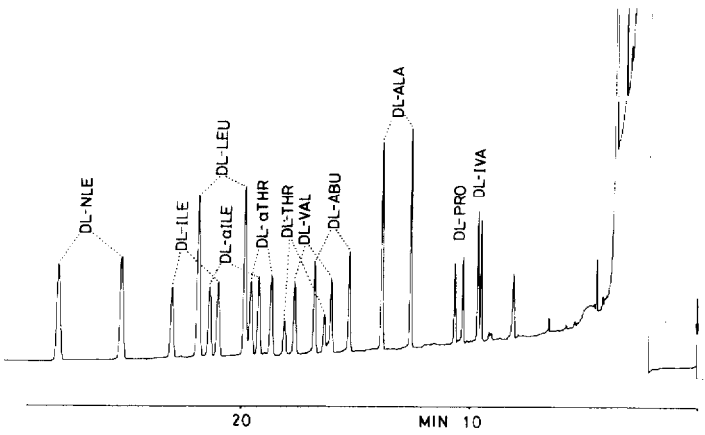


Fig. 1. Separation of some N-*tert.*-butylureido-D,L-amino acid isopropyl esters on a 48-m fused-silica capillary column of XE-60-L-valine-(*S*)-α-phenylethylamide. Column temperature, 180°C; carrier gas, hydrogen at 1.2 bar.

may be explained by the steric requirements of the *tert.*-butyl groups. The derivatives of most of the common α-amino acids can be obtained in good yields. We have not yet investigated basic amino acids, such as ornithine, lysine or histidine, and we believe that this method would not be applicable to arginine. Hydroxy groups of serine or threonine were trimethylsilylated after the formation of ureido derivatives. The separation factors of all the amino acid enantiomers investigated so far are listed in Table I. On the chiral polysiloxane XE-60–L-valine-(*S*)-α-phenylethylamide the D-enantiomers are eluted first (see Fig. 1). The derivatization proceeds without noticeable racemization, as proved by investigating pure enantiomers.

N-Methylamino acids

N-Methylamino acids are frequently encountered as constituents of peptide antibiotics and recently these modified amino acids have gained importance in peptide synthesis. They induce stability against proteolytic enzymes, a fact which may be of interest in connection with peptides with pharmacological properties. It has also been observed that N-methylamino acids tend to racemize to a much larger extent than the usual amino acids during peptide synthesis[9].

Configurational studies of N-methylamino acids are possible with diastereomeric (+)-3-methyl-2-butyl esters, as demonstrated in a recent study[15].

There is a special problem involved in the formation of volatile derivatives of N-methylamino acids for enantiomer separation. When the esters are submitted to trifluoroacetylation, heterocyclic alkylideneoxazolidine-5-ones are formed with concomitant complete racemization[16]. Even when the desired derivatives were obtained, they could not be separated into enantiomers.

The reaction of isocyanates with N-methylamino acid esters yields ureido derivatives which are only partially separated on XE-60–L-valine-(*S*)- or -(*R*)-α-phenylethylamide. However, when isocyanates react with free N-methylamino acids at elevated temperatures, the carboxylic group is converted into an N-alkylamide group with simultaneous conversion of the N-methyl group to an N-alkylureido derivative (Fig. 2)[8].

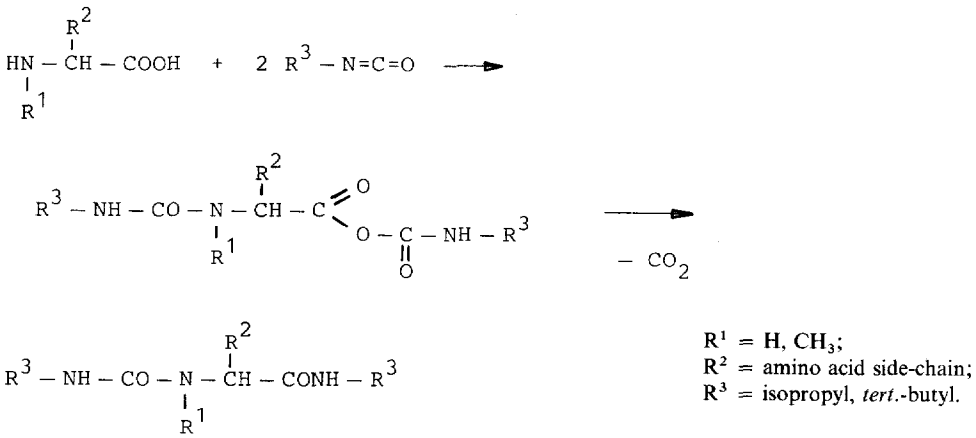
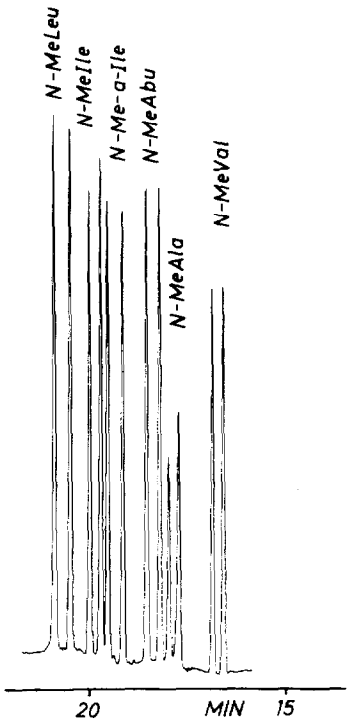


Fig. 2. Reaction of amino acids and N-methylamino acids with isocyanates at elevated temperatures.



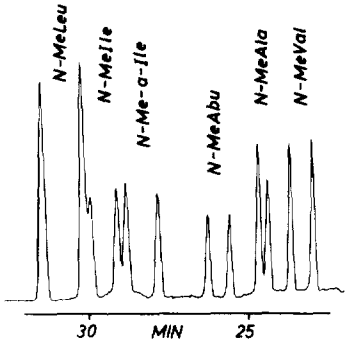


Fig. 3. Separation of N-methyl-D,L-amino acids as N-*tert.*-butylureido/N-*tert.*-butylamide derivatives on a 25-m Pyrex glass capillary column with XE-60–L-valine-(*R*)-α-phenylethylamide. Column temperature, 170°C; carrier gas, hydrogen at 0.6 bar. The L-enantiomers are eluted first.

Fig. 4. Separation of N-methyl-D,L-amino acids as N-isopropylureido/isopropylamide derivatives. Column and chromatographic parameters as in Fig. 3.

The availability of an extra amide group sufficiently increases the enantioselective interaction with the stationary phase to obtain complete enantiomer separation. The best separation was observed with N-*tert.*-butylureido/N-*tert.*-butylamides on XE-60–L-valine-(*R*)-α-phenylethylamide (Fig. 3).

However, it was found that the formation of *tert.*-butylamides gives rise to up to 40 % racemization. This can possibly be circumvented by forming the amides in the conventional way by aminolysis of an ester.

The reaction of N-methylamino acids with isopropyl isocyanate proceeds without noticeable racemization, and the corresponding N-isopropylureido/N-isopropylamides are formed in excellent yields with sample amounts as low as 100 μg. The best yields are obtained by heating a sample in equal amounts of isocyanate and dichloromethane for 15 min at 100°C in a tightly closed screw-capped vial. A separation of some standard derivatives on XE-60–L-valine-(*R*)-α-phenylethylamide is shown in Fig. 4; the separation factors are listed in Table II. The D-enantiomers are eluted after the L-enantiomers, in contrast to normal amino acids.

TABLE II

SEPARATION FACTORS (α) FOR THE SEPARATION OF N-METHYL-N-*tert.*-BUTYL-UREIDO-D,L-AMINO ACID *tert.*-BUTYLAMIDES (A) AND N-ISOPROPYLUREIDO-N-METHYL-D,L-AMINO ACID ISOPROPYLAMIDES (B) ON A 25-m PYREX GLASS CAPILLARY COLUMN WITH XE-60-L-VALINE-(*R*)-α-PHENYLETHYLAMIDE

L-Enantiomers are eluted first.

Amino acid	α *(A)*	*Column temperature (°C)*	α *(B)*	*Column temperature (°C)*
N-Me-Val	1.037	160	1.038	170
N-Me-Ala	1.023	160	1.014	170
N-Me-Abu	1.031	160	1.032	170
N-Me-*a*-Ile	1.033	160	1.037	170
N-Me-Ile	1.028	160	1.047	170
N-Me-Leu	1.037	160	1.049	170
N-Me-Phe*	1.009	160	1.000	180
N-Me-Hph**	1.008	160	1.007	180

* D-Enantiomer is eluted first. Separation factor on a 25-m capillary column of XE-60–L-valine-(*S*)-α-phenylethylamide = 1.012.

** N-Me-Hph = N-methylhomophenylalanine. Separation factor on a 25-m capillary column of XE-60–L-valine-(*S*)-α-phenylethylamide = 1.017.

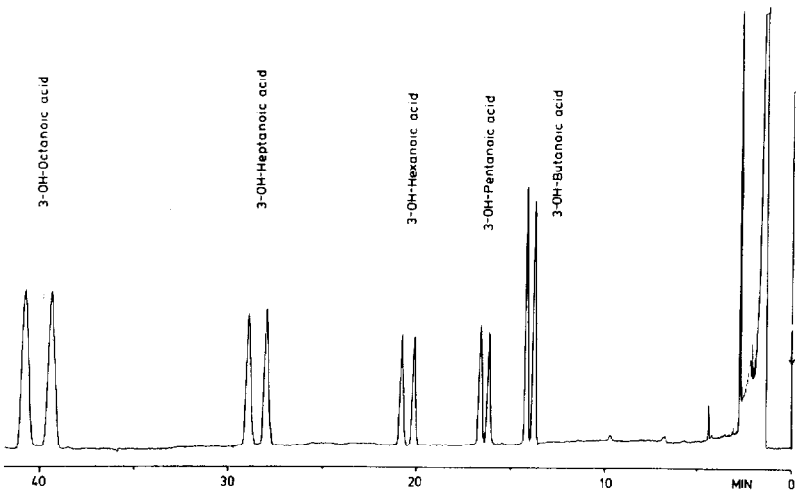


Fig. 5. Separation of 3-hydroxy acids as N-*tert.*-butyl carbamate/*tert.*-butylamide derivatives on a 25-m fused-silica capillary column of XE-60–L-valine-(*S*)-α-phenylethylamide (Chrompack). Column temperature, 180°C; carrier gas, hydrogen at 0.6 bar. The (*S*)-enantiomers are eluted first.

TABLE III

SEPARATION FACTORS (α) AND OPERATING TEMPERATURES FOR THE SEPARATION OF 3-HYDROXY ACIDS AS N-*tert.*BUTYL-CARBAMATE/*tert.*BUTYLAMIDE DERIVATIVES (A) AND N-ISOPROPYLCARBAMATE/ISOPROPYLAMIDE DERIVATIVES (B) ON A 25 m FUSED-SILICA CAPILLARY COLUMN OF XE-60–L-VALINE-*(S)*-α-PHENYLETHYLAMIDE

The (*S*)-enantiomers are eluted first.

3-Hydroxy acid	α *(A)*	*Column temperature (°C)*	α *(B)*	*Column temperature (°C)*
3-Hydroxybutanoic acid	1.030	180	1.022	180
3-Hydroxy-*n*-pentanoic acid	1.029	180	1.016	180
3-Hydroxyisohexanoic acid	1.025	180	1.008	190
3-Hydroxy-*n*-hexanoic acid	1.035	180	1.027	190
3-Hydroxy-*n*-heptanoic acid	1.044	180	1.031	180
3-Hydroxy-*n*-octanoic acid	1.032	190	1.022	190
3-Hydroxy-*n*-nonanoic acid	1.026	210	1.022	210
3-Hydroxy-*n*-decanoic acid	1.026	210	1.021	200
3-Hydroxy-*n*-tetradecanoic acid	–	–	1.018	220

3-Hydroxy acids

3-Hydroxy acids are widespread in nature. They have been found as constituents of several peptide antibiotics, *e.g.*, imacidin[17] and herbicolin A[18], as constituents of lipopolysaccharides[19], as natural metabolites of higher organisms[20] and as natural germination inhibitors in ants of the *Myrmicinae* family[21].

The carbamate derivatives of 3-hydroxy acid esters cannot be completely separated on XE-60–L-valine-(*S*)- or -(*R*)-α-phenylethylamide. The same problem occurs when diastereomeric derivatives were used for the separation of 3-hydroxy acids[22]. By application of isocyanates as reagents, N-alkylcarbamate/alkylamide derivatives are obtained. With these derivatives it is now possible for the first time to separate these enantiomers. The best separations are obtained with *tert.*-butyl isocyanate as reagent (Fig. 5). The separation factors of *tert.*-butyl and isopropyl derivatives are listed in Table III.

For assigning the order of elution of the derivatives of 3-hydroxy acids, it was necessary to prepare optically active reference samples. This was achieved by enzymatic reduction of β-keto esters with yeast, by analogy with the preparation of 3-hydroxybutanoic acid by Mori and Tanida[11]. We found that in this stereoselective reaction either the (*R*)- or the (*S*)-enantiomers of 3-hydroxy acid esters are formed in excess, as concluded from the positive or negative optical rotation, which can be used for correlation. The (*R*)-enantiomers of the carbamate/amide derivatives are consistently eluted after the (*S*)-enantiomers from a column of XE-60–L-valine-(*S*)-α-phenylethylamide.

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