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Note

Configurational analysis and test of racemization of N-methylamino acids by capillary gas chromatography

WILFRIED A. KÖNIG*, INGRID BENECKE and JÜRGEN SCHULZE
Institut für Organische Chemie und Biochemie der Universität, D-2000 Hamburg 13 (G.F.R.)
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Although N-methylamino acids are frequently encountered in natural compounds, especially as constituents of peptide antibiotics[1,2], the identification of their configuration has so far been possible only by comparison of their optical rotation with that of reference compounds[2] or by NMR spectroscopy[3]. An even more difficult problem is the determination of the degree of racemization of N-methylamino acids during various steps of peptide synthesis. It has been reported that N-methylamino acids give significant racemization during peptide bond formation[4,5].

For configurational analysis of amino acids sensitive gas chromatographic methods have been developed. Direct separation of enantiomers on optically active stationary phases has proved to be a particularly valuable tool[6–9]. Esterification and N-acylation are necessary for converting amino acids into volatile derivatives suitable for separation of enantiomers. When these reactions are applied to N-methylamino acids the expected derivatives are not obtained, but an intramolecular redox reaction occurs and cyclic alkylidene-oxazolidin-5-ones are formed[10]. In these compounds the hydrogen atom of the asymmetric centre is rearranged to yield a racemate of the heterocyclic compound. The enantiomers of N-methylamino acid esters without an N-acyl group are not separated on chiral phases.

In this paper we describe a procedure for the separation of diastereomeric (+)-3-methyl-2-butyl esters of N-methylamino acids by capillary gas chromatography.

EXPERIMENTAL

Materials

N-Methylamino acids were prepared from L- and DL-amino acids according to the procedure of McDermott and Benoiton[11]. (+)-3-Methylbutan-2-ol was prepared as described by Halpern and Westley[12]. Reagents for trimethylsilylation and for deactivation of capillary columns were purchased from Regis Chemical Co. (U.S.A.) and Machery, Nagel & Co. (Düren, G.F.R.).

Formation of derivatives

The (+)-3-methyl-2-butyl esters of N-methylamino acids were obtained according to the procedure described previously[13].

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Gas chromatography

For the separation of diastereomeric esters of N-methylamino acids 25-m fused silica and Pyrex glass columns coated with CpSil-5 (Chrompack, Berlin, G.F.R.) and SE-30AMAC (Franzen Analysentechnik, Bremen, G.F.R.) and a Model 2101 gas chromatograph (Carlo Erba, Milan, Italy), were used.

RESULTS AND DISCUSSION

The separation of diastereomeric derivatives has been suggested as an alternative to direct separation of enantiomers. We have shown that N-acylated amino acid (+)-3-methyl-2-butyl esters are well separated on capillary columns[13]. The precision of this method depends mainly on the optical purity of the chiral reagent. (+)-3-Methylbutan-2-ol can be prepared in about 99% optical purity.

Gas chromatography of N-methyl-DL-amino acid (+)-3-methyl-2-butyl esters gave unsymmetrical, tailing peaks and incomplete separations, even on highly deactivated fused silica capillaries. We therefore tried to reduce the polarity of the derivatives by N-trimethylsilylation. Gas chromatographic–mass spectrometric investigation of the reaction products, however, revealed that not one but a mixture of products was obtained, consisting mainly of unreacted N-methylamino acid ester, some N-silylated derivative and some N-methyl-N-trimethylsilylamino acid trimethylsilyl ester. The N-methyl-N-trimethylsilyl-DL-amino acid (+)-3-methyl-2-butyl esters were not completely separated in the case of N-methylalanine and N-methylleucine. Complete separations were observed for N-methylphenylalanine, N-meth-

Fig. 1. Separation of (+)-3-methyl-2-butyl esters of DL-valine, N-methyl-DL-α-aminobutyric acid and N-methyl-DL-valine on a 25 m × 0.23 mm I.D. fused silica capillary column coated with CpSil-5. Column temperature, 90°C. Carrier gas, hydrogen (0.6 bar). Co-injection of 0.5 μl of MSTFA.

Fig. 2. Separation of (+)-3-methyl-2-butyl esters of N-methyl-DL-leucine, N-methyl-DL-*allo*-isoleucine and N-methyl-DL-isoleucine. Column as in Fig. 1. Column temperature, 100°C. Co-injection of 0.5 μl of MSTFA.

TABLE I

SEPARATION FACTORS (α) AND OPERATING TEMPERATURES FOR SEPARATION OF N-METHYL-DL-AMINO ACID (+)-3-METHYL-2-BUTYL ESTERS (A) AND N-METHYL-N-TRIMETHYLSILYL-DL-AMINO ACID-(+)-3-METHYL-2-BUTYL ESTERS (B) ON A 25 m PYREX GLASS CAPILLARY COLUMN COATED WITH CpSIL-5

Co-injection of 0.5 μl of MSTFA.

Racemate	*A*		*B*	
	α	*Column temperature (°C)*	α	*Column temperature (°C)*
N-Methylalanine	1.038	80	1.018	100
N-Methylaminobutyric acid	1.041	80	Not tested	–
N-Methylvaline	1.030	100	1.050	110
N-Methylleucine	1.024	100	1.015	120
N-Methylisoleucine	1.032	100	1.038	120
N-Methyl-*allo*-isoleucine	1.026	100	Not tested	–
N-Methylphenylalanine	Not tested	–	1.046	160

ylvaline and N-methylisoleucine. In all these instances the D-enantiomer has a longer retention time than the L-enantiomer.

The most surprising result was that the unchanged N-methyl-DL-amino acid (+)-3-methyl-2-butyl esters were now eluted as symmetrical and completely resolved peaks, the L-enantiomer having the longer retention time (Figs. 1 and 2 and Table I). The same result could be obtained when 0.5 μl of the silylating reagent was co-injected with 1 μl of a solution of N-methyl-DL-amino acid (+)-3-methyl-2-butyl esters in methylene chloride. This effect was independent of the type of silylating agent when using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), N,O-bistrimethylsilyltrifluoroacetamide (BSTFA), hexamethyldisilazane (HMDS), N-methyl-N-trimethylsilylheptafluorobutyramide (MSHFBA), N,N-dimethyl-N-trimethylsilylamine (TMSDMA) and N,O-bistrimethylsilylacetamide (BSA). Only

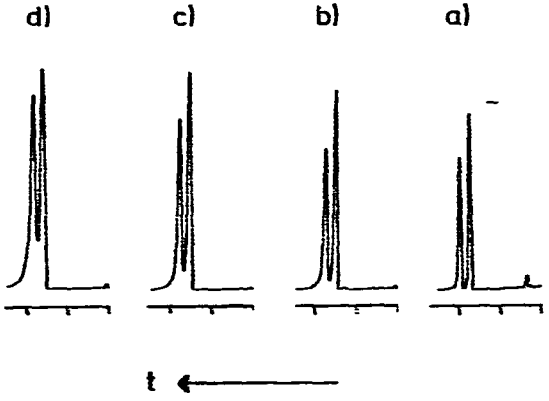

Fig. 3. Separation of (+)-3-methyl-2-butyl esters of N-methyl-DL-valine. (a) Co-injection of 0.5 μl of MSTFA; (b) re-injection without MSTFA after 10 min; (c) after 20 min; (d) after 40 min.

trimethylchlorosilane (TMCS) was ineffective. The deactivation phenomenon is only observed on co-injection and cannot be achieved with equal quality by injection of a silylating agent more than 10 sec before or after injection of the sample. The deactivating effect also is only temporary, as demonstrated in Fig. 3. This indicates that the molecules of the silylating agent cover the active centres of the inner surface of the column by adsorption rather than reacting chemically with free Si–OH groups still present. This hypothesis is also supported by the observation that the deactivation effect decreases markedly at temperatures above 120°C.

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