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Note

Chromatographic procedures for the identification of isomeric methylprolines

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Several methylprolines have been isolated from natural sources. *cis*-3-Methyl-L-proline occurs in the peptide antibiotic bottromycin¹. *trans*-4-Methyl-L-proline is found in apples^{2,3}, in the monamycins⁴ and in griselimycin⁵. *cis*-4-Methyl-L-proline was identified⁶ in hydrolyzates of antibiotic ICI 13,959. Recently, we have identified⁷ *cis*-5-methylproline as a component of actinomycin Z₅. In addition, 4-methylproline, and to a lesser extent 5-methylproline, when added to culture media of *Streptomyces antibioticus*, are incorporated into the actinomycins produced by that organism⁸. As a result of these and other studies⁹, a few reports concerning the chromatography of the various methylprolines can be found in the literature. A paper chromatographic study of cyclic imino acids has been reported¹⁰. Direct comparisons of all of the isomeric methylprolines by paper, ion-exchange and gas-liquid chromatography are described in this paper.

EXPERIMENTAL

Apparatus

High-voltage paper electrophoresis was effected with an apparatus from Gilson, Middleton, Wisc., using 4% formate buffer, pH 1.9, at 4800 V for 3 h. The amino acid analyzer was a Beckman Spinco Model 120C, with 0.2 M sodium citrate buffer, pH 3.05, flow-rate 34 ml/h. For gas-liquid chromatography (GLC) a Shimadzu Model 4BM chromatograph equipped with flame ionization detectors was used. The carrier gas was argon and the glass columns were 6 ft. × 3 mm I.D. Column A contained 3% EGSP-Z on Gas-Chrom Q (100-120 mesh) and column B 0.5% EGA on Chromosorb W (60-80 mesh).

Materials and methods

All of the amino acids were racemic except where otherwise stated. The *cis*- and *trans*-3-methylprolines¹¹ and 4-methylproline¹² (*cis-trans* mixture) were syn-

thesized as reported previously. *cis*-4-Methyl-D-proline was supplied by Prof. G. W. Kenner, Liverpool University, Great Britain. *cis*-5-Methylproline was supplied by Dr. H. Gershon, Boyce Thompson Institute, Yonkers, New York, U.S.A. 5-Methylproline (*cis-trans* mixture) was prepared from the *cis*-isomer by epimerization in 25% acetic anhydride-acetic acid (15 min at 150°), followed by hydrolysis with 2 *N* HCl (6 h at 110°).

The paper chromatographic solvent systems were (1) *n*-butanol-acetic acid-water (4:1:5); (2) methanol-water-pyridine (20:5:1) (3) *n*-butanol-phenol-acetic acid-water (3:1:1:5). The spots were rendered visible with ninhydrin (yellow) and isatin (blue) reagents.

Amino acid derivatives for GLC were prepared as follows. The amino acid (1 mg) was treated with 10% thionyl chloride-methanol (0.6 ml) at 80° for 1 h in a sealed tube. In order to obtain N-trifluoroacetyl methyl esters¹³, a portion was evaporated to dryness and the residue treated with 10% trifluoroacetic anhydride-dichloromethane (0.1 ml) at 55° for 1 h. In order to obtain N-acetyl methyl esters, a portion was evaporated to dryness and the residue treated with 20% acetic anhydride-pyridine at 25° for 2 h.

RESULTS AND DISCUSSION

The separation of the various methylprolines using the amino acid analyzer is depicted in Fig. 1, and their retention times in comparison with that of proline are given in Table I. Only 4-methylproline failed to give a diastereoisomeric separation. The remarkable separation of *cis*- and *trans*-3-methylprolines was observed earlier¹¹. Some data from paper chromatographic and electrophoretic experiments are also given in Table I. These techniques separate the methylprolines from proline but are not very effective for separating the various methylprolines.

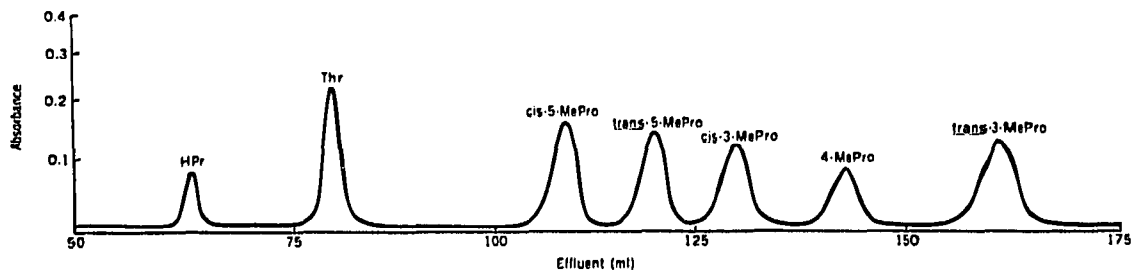


Fig. 1. Separation of the isomeric methylprolines with the amino acid analyzer (hydroxyproline and threonine are included as standards).

GLC retention times obtained with N-trifluoroacetyl and N-acetyl methyl esters are given in Table II. Diastereoisomeric separation was observed for all of the acetyl derivatives, and for all of the trifluoroacetyl derivatives except that of 5-methylproline. In all instances the *cis*-isomer had a longer retention time than the *trans*-isomer. This technique serves to distinguish all of the isomeric methylprolines from each other, and can be combined with mass spectrometry⁷ for the identification of methylprolines from natural sources.

TABLE I

COMPARISON OF PROLINE AND METHYLPROLINES USING THE AMINO ACID ANALYZER (AAA), PAPER ELECTROPHORESIS (PE) AND PAPER CHROMATOGRAPHY (PC)

Paper electrophoretic mobilities are expressed relative to sarcosine= 1.00. Paper chromatographic R_F values refer to solvent systems 1, 2 and 3 (see *Materials and methods*).

Amino acid	AAA (min)	PE	PC ₁	PC ₂	PC ₃
Proline	202	0.78	0.30	0.62	0.37
<i>cis</i> -3-Methylproline	229	0.74	0.38	0.68	0.52
<i>trans</i> -3-Methylproline	280	0.77	0.41	0.67	0.56
<i>cis</i> -4-Methylproline	250	} 0.69	0.42	0.69	0.53
<i>trans</i> -4-Methylproline	250				
<i>cis</i> -5-Methylproline	191	} 0.72	0.39	0.70	0.53
<i>trans</i> -5-Methylproline	211				

TABLE II

RETENTION TIMES (min) OF N-TRIFLUOROACETYL (TFA) METHYL ESTERS AND N-ACETYL (Ac) METHYL ESTERS ON GAS CHROMATOGRAPHIC COLUMNS A AND B

Amino acid	TFA on A (110°)	TFA on B (110°)	Ac on A (150°)	Ac on B (140°)
Proline	13.7	12.8	13.1	12.1
<i>cis</i> -3-Methylproline	13.8	13.9	13.6	12.8
<i>trans</i> -3-Methylproline	12.0	12.3	12.3	11.8
<i>cis</i> -4-Methylproline	14.7	16.5	14.6	14.6
<i>trans</i> -4-Methylproline	13.3	14.4	13.1	13.2
<i>cis</i> -5-Methylproline	11.9	12.2	11.5	10.8
<i>trans</i> -5-Methylproline	11.9	12.2	10.9	9.9

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