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SEPARATION OF HYDROXYAMINO ACIDS AND DETERMINATION OF THEIR CONFIGURATION BY GAS-LIQUID CHROMATOGRAPHY.

M.Ya.Karpeisky, S.V.Shlyapnikov, V.S.Oseledchik.
Institute of Molecular Biology, USSR Academy of
Sciences, Moscow, USSR

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Different isomers of β-hydroxy-α-amino acids are known to be constituents of many natural and synthetic biologically active compounds (1).

There are some publications on separation of certain amino acid diastereoisomers by gas-liquid chromatography using N-trifluoroacetylamino derivatives (2-3).

The purpose of this paper is to show the application of gas-liquid chromatography for studying the configuration of the diastereoisomers of hydroxyamino acids.

The method presented here is based upon the differences in physico-chemical properties of diastereoisomers depending upon the structure of substituent groups at the asymmetric centre.

The formation of inter- and intra-molecular hydrogen bonds necessarily accompanies the gas-liquid chromatography of mono-N-trifluoroacetyl /TFA/ methyl esters of β-hydroxy-α-amino acids, since these derivatives possess a free hydroxyl group. It is evident that a change in the nature of the functional group in one of the asymmetric centres, e.g. as a result of acylation of the hydroxyl groups (4), should exert a profound effect on the interaction of a compound with a stationary phase and consequently on the separation of threo- and erythro-isomers.

Quantitatively this effect for each amino acid may be expressed by the ratio of retention volumes of the compound having free and acylated hydroxyl groups /V^{rel}/. One may expect that this value is different for the threo- and erythro-isomers and constant for the compounds of the same stereochemical series within the same chemical class.

In Table 1 the retention times are presented for some amino acid diastereoisomers chromatographed as methyl esters of mono-N-TFA /Fig. 1/ (5) and N,O-bis-TFA-derivatives /Fig. 2/ (6) on a straight column /120 x 0,4 cm./ packed with 0,5 % polyethylenglycoladipate on Chromosorb W /60-80 mesh/ in acid form (7).

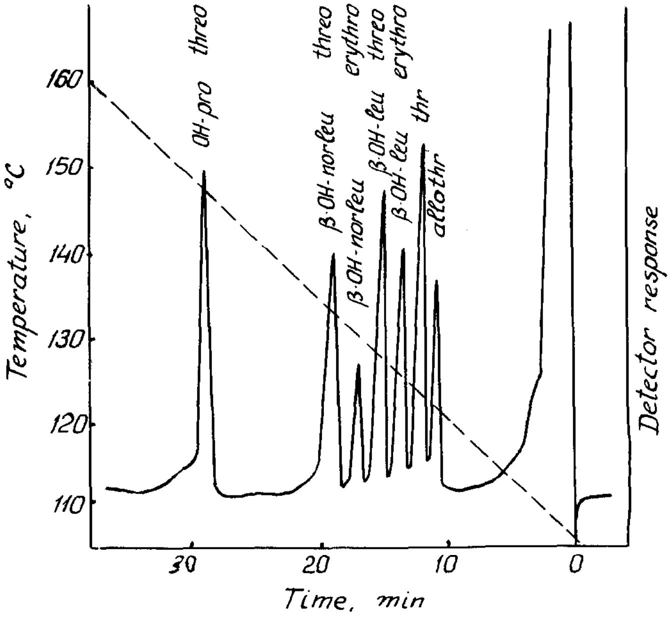

FIG. 1

Chromatographic separation of methyl esters of mono-N-TFA-derivatives. The rate of gas-carrier flow is 75 ml/min. The rate of temperature increase is 1,3°C per minute.

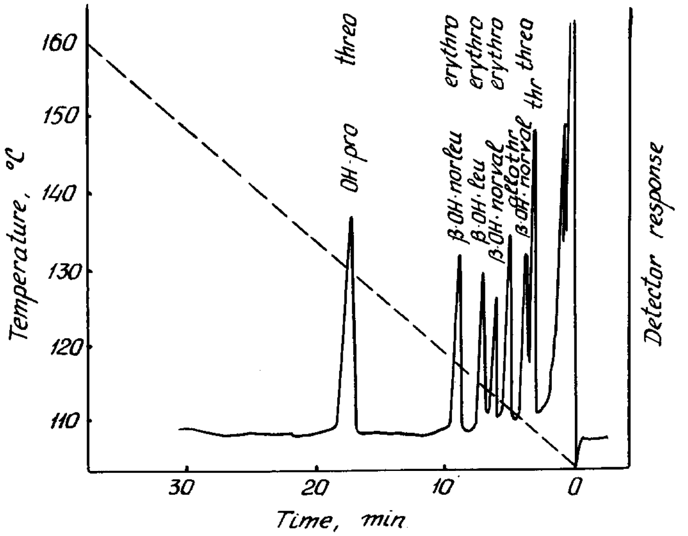

FIG. 2

Chromatographic separation of methyl esters of N,O-bis-TFA-derivatives. The rate of gas-carrier flow is 75 ml/min. The rate of temperature increase is 1,3°C per minute.

TABLE 1 (8)

Hydroxyamino acid		Retention time /min/		
		mono-N-TFA	N,O-bis-TFA	$V_{rel} = \frac{\text{mono-N-TFA}}{\text{N,O-bis-TFA}}$
			103°C	
β-hydroxy-α-aminobytiric acid	erythro-	16,1	6,9	2,33
	threo-	19,1	4,3	4,45
β-hydroxy-α-aminovaleric acid	erythro-	22,2	9,6	2,31
	threo-	29,3	6,5	4,51
β-hydroxy-α-aminoisocaproic acid	erythro-	24,4	10,6	2,30
	threo-	29,3	6,5	4,51
β-hydroxy-α-aminocaproic acid	erythro-	34,1	14,8	2,30
	threo-	41,1	9,3	4,42
			140°C	
γ-hydroxyproline	threo-	15,2	3,0	5,06
			160°C	
β-hydroxy-β-phenyl-α-amino-propionic acid	erythro-	12,1	2,5	4,84
	threo-	14,0	2,4	5,85

Aliphatic β-hydroxy-α-amino acid diastereoisomers may be satisfactorily separated under these conditions; trifluoroacetylation of the hydroxy groups invers the sequence of elution of configurational isomers.

The results given in Table 1 show that the V^{rel} value of aliphatic hydroxyamino acid diastereoisimers with similar configuration stays constant. At the same time a marked difference is observed in absolute V^{rel} value for the threo- and erythro-isomers. However for gas-liquid chromatographic separation of diastereoisomers of some nonaliphatic hydroxyamino acids e.g., γ-hydroxyproline and phenylserine the absolute value of V^{rel} markedly differs from V^{rel} found for aliphatic β-hydroxyamino acids.

The results obtained demonstrate that the value of relative retention of mono-N-TFA and N,O-bis-TFA-methyl esters derivatives of amino acids is constant and specific for each class of hydroxyamino acids and thus serves as a criterion of the threo- or erythro-configuration of the isomers studied.

REFERENCES

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4. In the reactions of this type the asymmetrical carbon is not involved; therefore no change in original configuration of a compound occures.
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7. "PYE" chromatograph with an ionisation detector /20 mC Sr^{90} / was used in this investigation.
8. Chromatographic separation was conducted using a rate of gas-carrier /argon/ flow equal to 75 ml/min.