Determination of Stereochemical Configuration of Asymmetric 1-Methylalkylamines, *via* the Stereospecific Action of Leucine Aminopeptidase on Diastereoisomeric L-Leucyl-(±)-1-methylalkylamides

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THE advantage of enzymatic determination of absolute configuration over physicochemical measurements is that, once the stereospecificity of the biological system is known, racemic rather than optically pure materials can be used.[1]

Previous studies on the stereochemical specificity exhibited by leucine aminopeptidase toward DL-amino-acid amides, have always been confined to the α-asymmetric centre of the α-amino-acids involved and the enzyme was shown to be specifically L-directing.[2]

TABLE 1. *Relative susceptibility of* L-*leucyl-(±)-alkylamides to leucine aminopeptidase**

(±)-Amine from which substrate is derived†	Ratio of concentrations of unhydrolysed diastereoisomers $\frac{\text{L-(+)}}{\text{L-(−)}}$ after period of incubation (hr.) 0	1	2	3	4	6	8	24	G.l.c. analyses of *N*-trifluoroacetyl derivative‡ Separation temperature (°C)	Retention times (min.) L-(−)	L-(+)
1-Methylpropylamine ..	1·0	1·4			2·8			19	110	54·8	57·0
1-Methylbutylamine ..	1·0	2·3	11		102		∞		120	41·3	44·2
1-Methylhexylamine ..	1·0	1·9			2·4		17		130	38·3	41·3
1-Methylheptylamine ..	1·0		1·3			1·9		4·0	130	62·3	65·3
1,2-Dimethylpropylamine ..	1·0		1·3		1·5		2·5	11	122	35·3	39·0
1,3-Dimethylbutylamine ..	1·0	3·8	7·7		98		∞		115	49·5	54·0
1,4-Dimethylpentylamine ..	1·0	1·3		2·2				20	115	78·8	86·3
1,5-Dimethylhexylamine ..	1·0			1·3		1·8		5·0	130	48·2	52·5
α-Methylphenethylamine ..	1·0	31	120		∞				170	32·5	36·0

* Commercial preparations of leucine aminopeptidase from a number of suppliers were used. (1 mg. of enzyme hydrolyzed approx. 170 μmole of leucine amide per min.)

† The substrates were prepared from benzyloxycarbonyl-L-leucine hydrazide and the (±)-amine *via* the azide (ref. 3) followed by the removal of the benzyloxycarbonyl group by hydrogenation. The compounds were used without further purification, as attempts to recrystallise the reaction products resulted in partial resolution of the diastereoisomeric mixture. Before use in the assay, the substrates were shown to be chemically pure by t.l.c. and all compounds were characterized by mass spectrometry. In the same way, the L-leucyl-(−)-1-methylalkylamides were prepared from optically pure (−)-1-methylalkylamines, obtained by resolution *via* the (+)-tartrates.

‡ G.l.c. analyses were carried out on a Wilkens Autoprep gas chromatograph using a 15 ft. × ¼ in. column (0·75/0·25 w/w % of DEGS/EGGS-X on Chromosorb W). During the analyses the nitrogen flow was 60 ml./min.

We have now observed that the hydrolytic action of leucine aminopeptidase on a series of diastereoisomeric L-leucyl-(±)-1-methylalkylamides always results in a preferential hydrolysis of the L-leucyl-(−)-1-methylalkylamide. A sensitive g.l.c. technique was used to determine the respective rates of metabolism of the diastereoisomeric pairs or, alternatively, the stereospecific action of the enzyme could be qualitatively demonstrated by a simple t.l.c. procedure.

In a typical experiment, the substrate (0·4 mmole) in aqueous methanol (4 ml. of 40%), an internal standard [D-alanine-(+)-menthyl ester pTS (0·0075 mmole)], tris buffer [5 ml. of 0·1M, containing $MnCl_2$ (0·002M) and leucine aminopeptidase (0·05 mg. protein in 0·5 ml.) were incubated at 37°. From time to time aliquots (2 ml.) were removed and cooled to 0° and then immediately saturated with solid K_2CO_3. After extracting with ethyl acetate (8 × 5 ml.), drying ($MgSO_4$), and concentrating to 5 ml., methyltrifluoracetate (1·5 ml.) and triethylamine (0·7 ml.) were added. After 30 min. at room temperature, the sample was washed (dil. HCl and H_2O), dried ($MgSO_4$), and concentrated to 0·5 ml. and a part of the solution (~2 μl) injected into the gas chromatograph. By computing the peak areas of the diastereoisomers relative to that of the internal standard, a fast sensitive recording of unhydrolysed diastereoisomer concentration could be obtained (Figure). When expressed as a ratio of $\frac{\text{L-(+)}}{\text{L-(−)}}$ this gave a measure of the antipodal specificity of the enzyme. Although there was considerable variation in the rate of attack on the substrates,

only in the case of L-leucyl-(±)-α-methylphenethylamide was there any noticeable hydrolysis of the L-(+)-isomer.

The asymmetrical hydrolysis can also be followed by spotting the reaction mixture directly on to silica gel "G" plates, developing, and spraying with ninhydrin.

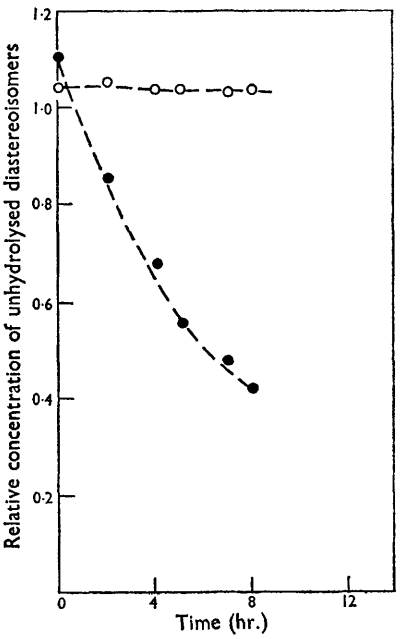
TABLE 2

Thin-layer chromatography of diastereoisomeric L-leucyl-(±)-alkylamides,* $Me_2CH{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO{\cdot}NHR$

R=	R_f-Values of diastereoisomers L-(+)	L-(−)
1-Methylpropyl	0·48	0·55
1-Methylbutyl	0·48	0·60
1-Methylhexyl	0·54	0·68
1-Methylheptyl	0·55	0·71
1,2-Dimethylpropyl	0·50	0·58
1,3-Dimethylbutyl	0·50	0·60
1,4-Dimethylpentyl	0·55	0·69
1,5-Dimethylhexyl	0·55	0·70
α-Methylphenethyl	0·53	0·66

* Silica gel "G" (Merck). Solvent system BuOH–HCO_2H–H_2O (70:15:15).

Since a number of (+)-1-methylalkylamines have already been related to the absolute L-configuration of the α-amino-acids (ref. 4) we conclude that all (+)-1-methylalkylamines have the absolute L-configuration and that the rate of action of leucine aminopeptidase on L-leucyl-D-alkylamide is always faster than that on the corresponding L,L-diastereoisomer.



FIGURE

Hydrolysis of L-leucyl-(±)-1,2-dimethylpropylamide by leucine aminopeptidase.

○ *L-Leucyl-(+)-1,2-dimethylpropylamide.*

● *L-Leucyl-(−)-1,2-dimethylpropylamide.*

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