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RACEMISATION DURING PEPTIDE SYNTHETIC WORK - I
EFFECT OF SUBSTITUENT LINKED TO THE beta-CARBON ON BASE-CATALYSED RACEMISATION OF N-CARBOBENZOXY AMINO-ACID ACTIVE DERIVATIVES

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Relatively little is known about mechanisms of racemisation during peptide bond-forming reactions. Apart from oxazolone theory, the initial step in the racemisation may involve ionisation of the hydrogen atom linked to the asymetric carbon. Thus Young suggestes that unusual tendency to racemise in alkali found for active derivatives of N-carbobenzoxy-S-benzylcysteine[1] may be due to the lability of the alfa-hydrogen and stabilising of anion /IIa/ by resonance with the contributing form /IIb/.[2] Swan's suggestion is that this racemisation proceedes by complete elimination and readdition of benzyl mercaptan.[3]

In connection with our studies on beta-cyano-L-alanine[4] we wish to report further examples of rapid base-catalysed racemisation.

Some results are presented in Table I.

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1 B.Iselin, M.Feurer, R.Schwyzer, Helv. Chimica Acta 38, 1508 /1955/

2 G.T.Young, Proc. Peptide Symposium, Prague 1958; Coll. Czechoslovak Comm., Special Issue 24, 118 /1959/

3 J.A.Maclaren, W.E.Savige, J.M.Swan, Austral. J. Chem. 11, 345 /1958/

4 B.Liberek, Chem. and Ind. 987 /1961/; Bull. Acad. Pol. Sci., Ser. Sci. Chim. 10, 227, 407 /1962/

T A B L E I

All experiments carried out at 22.5°C ± 0.5
2% solutions containing in 100 ml 1.2 ml of N/Et/3
A-acetone solution, B-DMF solution, C-ethyl acetate solution

No	Compound	Time within optical rotation decreases to zero or near constant value	Isolated racemate or near racemised product		
			Yield % /crude/	analitical sample m.p. °C	$[\alpha]_D^{20}$ c=2.0 acetone
1	Z-Ala.ONp (CN)	A 3.5 hrs	52	116-7	0.0
2	Z-Ala.OPh[a] (CN)	A 3 days	92	119-120	0.0
3	Z-Ala.OMe (CN)	A	Within 15 days optical rotation decreases from -37.0 to -35.0		
4	Z-Asp.O⌐¬	A 1.5 hrs	90% of crude racemic N-carbobenzoxy aspartic acid was obtained		
5	Z-Asp.ONp (OBz)[b]	A 2 days	67	97-98	0.0
6	Z-Cys.ONp (SBz)	A 26 hrs	58	93-95 /100/[c]	-1.8[d]
7	Z-Cys.OCH_2-CN[1] (SBz)	A 6 days B 10 days C 20 days	94 90 90	102-3 102-3 102-3	0.0 0.0 0.0
8	Z-Phe.ONp	A 11 days	48	104-7 /113/[c]	-4.7[e]
9	Z-Leu.ONp	A	Within 18 days optical rotation decreases from -30.8 to -15.4		

Symbols according to M.Goodman and G.W.Kenner, Adv. Protein Chem., 12, 465 /1957/

a.L-isomer m.p.138°C, $[\alpha]_D^{20}$ -60.4 /c=2.0 acetone/

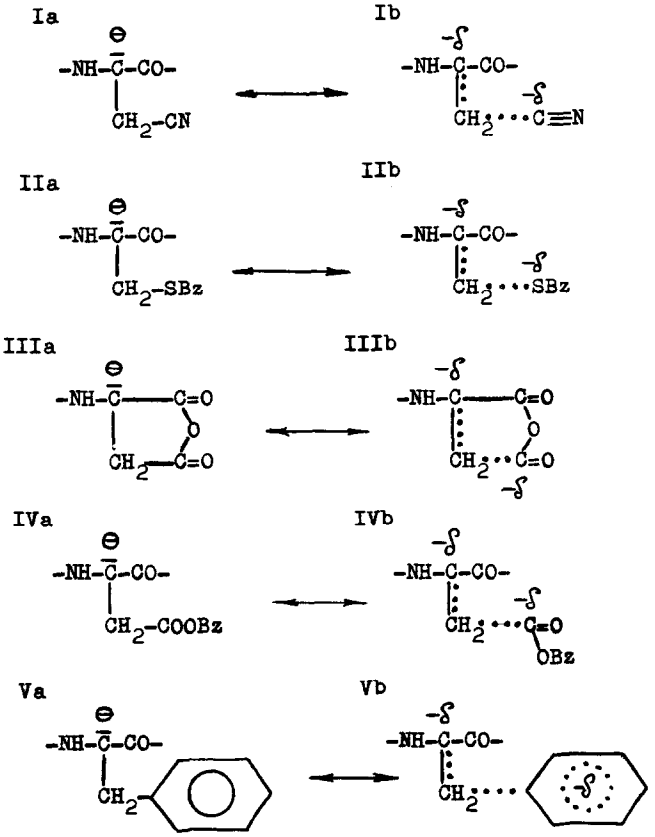
b.L-isomer m.p.79°C, $[\alpha]_D^{20}$ -23.1 /c=2.0 acetone/

c. in parenthesis complete clearing of the sample

d.L-isomer $[\alpha]_D^{20}$ -37.2 /c=2.0 acetone/; e.L-isomer $[\alpha]_D^{20}$ -18.2 /c=2.0 acetone/

For all new compounds satisfactory nitrogen analysis have been obtained.

A possible mechanism which may be pictured for this base-catalysed racemisation is presented below.



The initial step is proton abstraction from the asymetric centre /Ia,IIa,IIIa,IVa,Va/. The anion /Ia/ may be considerably stabilised by contribution of the anion /Ib/ in which the negative charge on the alfa-carbon is coniugated with π electrons of beta-situated cyano group. Thus active derivatives of N-carbobenzoxy amino-acids which have <u>in beta position a group capable of involving a type of coniugation with the negative charge on the alfa-carbon would racemise easily in alkali</u>.

This accounts especially for active derivatives of beta-cyanoalanine /Ib/, S-benzylcysteine /IIb/, aspartic anhydride /IIIb/, beta esters of aspartic acid /IVb/ and even aromatic or heterocyclic amino-acids /Vb/.

Lability of the alfa-hydrogen is dependent upon protection of the amino group and upon carboxyl activation for peptide synthesis. "Strong" activation leads to enhanced lability of the alfa-hydrogen /exp.1,2,3, Table I/ Base-catalysed racemisation might proceede by ionisation followed by mere enolisation $-NH-C(\bar{O}^{\ominus})=C-$ but this hypothesis without stabilising effect of beta-substituent seems insufficient for any real danger of loss of optical activity during peptide synthesis /exp.9, Table I/.

It is known that N-carbobenzoxy group protects amino-acids to whom it is attached against racemisation during peptide bond-forming reactions.[5] Bodanszky was the first to point out that this protective power is not an absolute but a relative one.[6] In our opinion formation of some peptide bonds is specially beset with danger of base-catalysed racemisation. Such cases preserve special attention even if stepvise approach for elongation of the peptide chain is applied[7] especially when hydrobromides from Ben Ishai procedure with triethylamine are used as the aminocomponent.

One would expect that protection of the thiol group of cysteine or beta carboxyl group of aspartic acid with electron releasing tert-butyl residue should minimize the danger of base-catalysed racemisation.

Base-catalysed racemisation of active derivatives of phthalyl amino-acids and racemisation during preparation of active cyanmethyl esters will be subiects to separatory communications.

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[5] G.T.Young, Proc.Peptide Symposium, Prague 1958, Coll.Czechslovak Comm.,Special Issue 24, 39 /1959/

[6] M.Bodanszky, C.A.Birkhimer, Chimia 14, 368 /1960/ M.Bodanszky, Ann.New York Acad.Sci. 655 /1960

[7] M.Bodanszky, V.du Vigneaud, J.Am.Chem.Soc. 81, 5688 /1959/