Tetrahedron Letters No. 22, pp. 1479-1483, 1963. Pergamon Press Ltd.
Printed in Great Britain.

RACEMISATION DURING PEPTIDE SYNTHETIC WORK - III.[+] PARTIAL RACEMISATION DURING PREPARATION OF ACTIVATED CYANOMETHYL ESTERS OF N-PROTECTED AMINO ACIDS.

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(Received 2 July 1963)

The activated cyanomethyl esters of N-protected amino acids are easily accessible by interaction of carboxylic acid and chloroacetonitrile in the presence of some excess of triethylamine.[1,2,3,4] Usually this esterification is carried out for 3 hours in boiling ethyl acetate as a solvent.[1,2,4] Such normal conditions employed for the ester formation are considered to give the products with maintenance of optical activity as the rule.[1,4,5] An exception, however, is N-carbobenzoxy-S-benzyl-L-cysteine yielding mainly racemic cyanomethyl ester, unless the reaction temperature is lower than usual.[2]

In view of previously reported evidence for base-catalysed racemisation[6,7] it seems probable that the normal conditions employed for the ester formation may be quite severe for certain N-protected amino acids. The cyanomethyl ester once formed should undergo base-catalysed racemisation, the rate of loss of optical purity being dependent upon the presence of an excess of triethylamine, upon the conditions of the esterification reaction, upon the structural specifity of particular amino acid and upon amino protection.

[+]Part II: Tetrahedron Letters No. 17, 1103 (1963).

TABLE I

A/ cyanomethyl ester prepared at room temperature, 4mM of N-protected amino acid, 8mM of $ClCH_2CN$ and 6mM of $N/Et/_3$; reaction time 18 hours

B/ cyanomethyl ester prepared at the b.p., 4mM of N-protected amino acid, 6mM of $ClCH_2CN$ and 6mM of $N/Et/_3$ in 6 ml of AcOEt; reaction time 5 hours

No	Compound	Conditions	Yield % crude product	Product crystallized from minimum amount of isopropanol or ethanol[a] m.p. °C	$[\alpha]_D$ /c=2.0 acetone/	Analytical sample /recrystallized/ m.p. °C
1	Z-Phe.OCH_2CN	A	70	52-54	-32.5	55-56
		B	74	54-79[b]	-24.0[b]	
2	Z-Ala.OCH_2CN	A	95	38.5-40[c]	-32.2	41-42[c]
		B	73	37-39[c]	-29.7	38.5-40[c]
3	Phth-Ala.OCH_2CN	A	91	115-117	-37.0	117-118
		B	94	88-90[d]	-1.8	90-91.5[d]
4	Phth-Phe.OCH_2CN	A	75	139-141	-206.0	142
		B	88	117-121[e]	-12.8	118-120[e]
5	Phth-Leu.OCH_2CN	A	94	73-76	-38.1	75-76.5
		B	96	68-71.5[f]	-22.0	69-72.5[f]
6	Phth-Ala(CN).OCH_2CN	A	69	117-120	-1.9	119-121
		B	68	116-120	0.0	121-122
7	Tos-Glu.OCH_2CN	A	73	143-144	-29.9	143.5-144.5
		B	72	143-144	-29.4	143.5-144.5
8	Benz-Tyr(OBenz).OCH_2CN	A	97	157-161	-75.8[g]	158.5-162
		B	98	185-189	-26.5[g]	190-191.5

Symbols according to Goodman and Kenner[5], benz = benzoyl, the cyanomethyl esters obtained as oils are not recorded in Table, all m.p. are uncorrected, optical rotations were measured at 18-22°, for all analytical samples satisfactory nitrogen analyses have been obtained;

Therefore, our previously reported results are now supplemented by the data, presented in Table I, on the extent of racemisation during preparation of cyanomethyl esters of those N-protected amino acids which are specially beset with the danger of base-catalysed racemisation.[6,7]

It is evident from the Table I that the cyanomethyl ester of N-carbobenzoxy-S-benzylcysteine is not the only curious example of partial racemisation.[2,5] All the results recorded in Table I can be explained in terms of base-catalysis with the initial proton abstraction from the alpha -carbon atom followed by formation of, or simple resonating with the contributing carbanions in which the asymmetry at C-alpha is lost.[6,7] Thus all phthaloyl amino acids including the phthaloyl-L-leucine are extensively racemised during "conversion" of carboxylic acid to its cyanomethyl ester.[7] Extensively racemised products resulted also from N-protected amino acids, in which the structural specifity of particular amino acid makes the alpha-hydrogen atom more labile due to stabilising of the carbanion by a type of coniugation involving the beta substituent and the negative charge at the alpha-carbon atom.[6] If the carbanion may be stabilized by the enol-like structures[7] and simultaneously by the coniugation effect involving the beta substituent,[6] exten-

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Footnotes to Table I /continued from the preceding page/

a/ some fractionation may have occur

b/ this product was separated in a lower melting, soluble in ethyl ether, optically active material m.p. 51-55° $[\alpha]_D$ -31.3 and a higher melting extensively racemised cyanomethyl ester m.p. 84-93° $[\alpha]_D$ -14.9; m.p. of authentic racemate 97-98°

c/ to the product dissolved in acetone benzine /b.p.125-140°/ was added and acetone evaporated to the first turbidity and then cooled and seeded

d/ m.p. of authentic racemate 91-92°

e/ m.p. of authentic racemate 119-120°

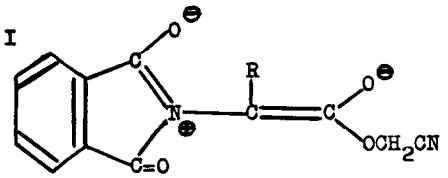
f/ m.p. of authentic racemate 79-80°

g/ c = 2.0 in dimethylformamide

sive racemisation may result even at room temperature and indeed this is the case with the phthaloyl-β-cyano-L-alanine which yields completely racemic product at the b.p. and mainly racemic product at room temperature /exp.6, Table I/.

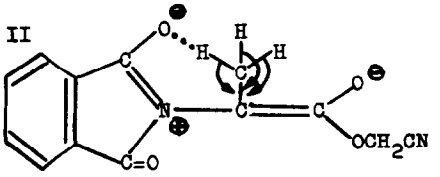
The lack of racemisation of cyanomethyl tosyl-L-pyroglutamate and the fact that cyanomethyl esters of phthaloyl amino acids are easily racemised may be worthy of attention from the standpoint of mechanism. Thus the loss of optical activity cannot be explained by the operation of the mere inductive effect of the two acyl groups attached to the nitrogen, but the most important factor is the stabilising coniugation, involving the benzene ring, in the enol-like contributing structures /I/.[7]

I



Viewing the unexpected tendency to racemisation in the cyanomethyl ester of phthaloyl-L-alanine /exp.3, Table I/ it seems likely that in this case the contributing enol-like structures /I/[7] might be additionally stabilised by the hyperconiugation effect /II/. This additional stabilisation conferred upon the enol-like species might be even strengthened by the electrostatic effect of the neighbouring negative charge /II/

II



The conclusion to be drawn from all the data is that retention of configuration is not always the rule during preparation of activated cyanomethyl esters. This would appear to be particularly significant since Williams and Young,[4] studying recently a stringent model reaction, found no racemate in the products of the cyanomethyl peptide bond-forming route.

Further, it seems probable that in addition to oxazolone hypothesis certain other mechanisms may be operating and can be considered for racemisation during peptide synthesis. This accounts even for carbobenzoxy amino acids especially when beta substituent might be involved in the stabilisation of the carbanion.[6]

Some confirmatory evidence for racemisation of carbobenzoxy amino acids during dipeptide synthesis has been reported lately by Geiger and coworkers[8] and by Weygand and coworkers.[9]

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