THE SYNTHESIS OF ALANYL- $\alpha$ -AMINOPROPIONITRILES(DL-DL, L-DL, and L-L) BY DEHYDRATING THE CORRESPONDING N-o-NITROPHENYLSULFENYLALANYL-ALANINE AMIDES

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N-o-Nitrophenylsulfenylalanylalanine amides(DL-DL, L-DL, and L-L) were dehydrated in POCl<sub>3</sub>-pyridine without affecting their N-protecting groups. The N-protected dipeptide nitriles obtained were treated with anhydrous HCl in ethyl acetate to give the hydrochlorides of the corresponding dipeptide nitriles. The results of ion exchange chromatography of these products indicated that the optical purity of the  $\alpha$ -aminopropionitrile group was well retained.

In the previous paper<sup>1)</sup>, some dipeptides containing  $\alpha$ -aminonitriles as their C-terminal residues were synthesized by coupling the active esters of N-tritylamino acids with  $\alpha$ -aminonitriles followed by detritylation with acids. DL-Alanyl--DL- $\alpha$ -aminopropionitrile which was not obtained in the results of the previous paper, has been synthesized by coupling N-o-nitrophenylsulfenyl-DL-alanine p-nitrophenyl ester with DL- $\alpha$ -aminopropionitrile followed by treating with HCl.<sup>2)</sup> However, it is more interesting to get peptide nitriles, which have optically active  $\alpha$ -aminonitrile groups, by the dehydration of the corresponding peptide amides. Woolley <u>et al.<sup>3)</sup></u> reported on the dehydration of N-protected dipeptide amides by means of POCl<sub>3</sub> in cold pyridine and obtained N-benzoyl-L-phenylalanyl-DL-serine nitrile etc. as intermediates for the synthesis of the corresponding amidines. However, the N-benzoyl group can not be removed without affecting the nitrile group.

In this study, DL-alanyl-DL-α-aminopropionitrile, L-alanyl-DL-α-aminopropionitrile, or L-alanyl-L-α-aminopropionitrile has been synthesized from N-o-nitrophenylsulfenyl(NPS)-DL-alanine and DL-alanine methyl ester, NPS-L-alanine and DLalanine methyl ester, or NPS-L-alanine and L-alanine methyl ester, respectively, as follows:

CHa HCl·H2N-CH-COOCH3 CH3 ÇH₃ CH₃ CH₃ NPS-NH-CH-CONH-CH-COOCH₃ NPS-NH-CH-COOH · DCHA (I) (II) M.p.(°C), Yield(%),  $[\alpha]_{D}^{22}(deg.)$ a: DL-DL 121-124 52 b: L-DL 112-114 58 140-142 c: L-L 53 -39.2 (c l,DMF)

	NI	H <sub>3</sub> -CH <sub>3</sub> OH	↔ NPS-NH-C	H <sub>3</sub> CH <sub>3</sub> H-CONH-CH-CO	)NH2	]	POCl <sub>3</sub> -Pyric	dine	<b>→</b>
				(III)					
				M.p.(°C), Y	ield	(%),[	$[\alpha]_D^{22}$ (deg.	)	
			a: DL-DL	164-166	38		D		
			b: L-DL	185-187	88				
			c: L-L	223-225	87	-	-31.3(c 0.	46,DMF)	
	NPS-	CH₃ -NH-CH-CONI	CH₃ H-CH-CN —	Anhydrous H		► НСІ	CH <sub>3</sub> L•H <sub>2</sub> N-CH-CO	CH₃ ONH-CH-CN	1
		(IV)					(V)		
		M.p.(°C),	Yield(%),	$[\alpha]_{D}^{22}(deg.)$			M.p.(°C),	Yield(%),	$[\alpha]_{D}^{22}(deg.)$
a:	DL-DL	145-147	50	Ľ	a:	DL-DL	195-197	28	D
b:	L-DL	165-167	45		b:	L-DL	(decomp.)		
c:	L-L	163 <b>-</b> 165	58	-61.6 (c 0.85,DMF)	с:	L-L			-86.0 (c 0.73, H <sub>2</sub> 0)
NP	S-; 0-1	Nitropheny]	lsulfenyl						

N-o-Nitrophenylsulfenylalanylalanine methyl ester(II-a, b, or c) was prepared by coupling the dicyclohexylammonium salt of NPS-alanine(DL or L) with the hydrochloride of alanine methyl ester(DL or L) by use of N,N-dicyclohexylcarbodiimide according to Zervas <u>et al</u>.<sup>4)</sup> The ester(II-a, b, or c) was easily converted into the corresponding NPS-dipeptide amide(III-a, b, or c) by ammonolysis in methanol saturated with ammonia at room temperature for at least two days. The optical purity of NPS-L-alanyl-L-alanine amide(III-c) was examined by ion exchange chromatography after removal of its N-protecting group with HCl. This dipeptide amide has only one peak whereas the other dipeptide amides have two peaks each due to the separation of the diastereomers as shown in Fig. l(dotted lines). It is known that dipeptide diastereomers can be separated into two peaks by ion exchange chromatography.<sup>5)</sup> Therefore no racemization occurred during the preparation of III-c.

The N-o-nitrophenylsulfenylalanylalanine amide(III-a, b, or c) was dehydrated in POCl<sub>3</sub>-pyridine.<sup>3,6)</sup> For this purpose the amide was dissolved in a small amount of pyridine and the solution was cooled in an ice salt bath below  $-5^{\circ}$ C. After POCl<sub>3</sub> had been added dropwise, the resulting solution was allowed to stand at room temperature for 30 minutes. Then ice was added to the solution to decompose the excess of POCl<sub>3</sub> and the solution was treated with ethyl acetate to extract the product. The N-o-nitrophenylsulfenylalanyl- $\alpha$ -aminopropionitrile(IV-a, b, or c) obtained was recrystallized from ethyl acetate-petroleum ether.

When the N-protected dipeptide nitrile(IV-a, b, or c) was treated with anhydrous HCl in ethyl acetate, the hydrochloride of alanyl- $\alpha$ -aminopropionitrile(V-a, b, or c) precipitated, which was recrystallized from ethanol-ether. The hydrochloride of DL-alanyl-DL- $\alpha$ -aminopropionitrile(V-a) crystallized easily, and the results of the elemental analysis of V-a agreed with the calculated values(Found: C, 39.73; H, 7.00; H, 23.34 %., calculated for C<sub>6</sub>H<sub>12</sub>N<sub>3</sub>OCl: C, 40.58; H, 6.81; N, 23.65 %). However, the hydrochloride of L-alanyl-DL- $\alpha$ -aminopropionitrile(V-b) and L-alanyl--L- $\alpha$ -aminopropionitrile(V-c) were very hygroscopic. Therefore their melting points and elemental analyses could not be determined. These two dipeptide nitriles(V-b and c) were identified by comparing their chromatograms with that of V-a, as shown in Fig. 1(solid lines).





DL-Alanyl-DL- $\alpha$ -aminopropionitrile and L-alanyl-DL- $\alpha$ -aminopropionitrile have also two peaks each due to the separation of the diastereomers. On the other hand, L-alanyl-L- $\alpha$ -aminopropionitrile has only one peak which corresponds to the latter peak of the above-mentioned diastereomers. This result indicates that no racemization occurred during the dehydration of III-c in POCl<sub>3</sub>-pyridine. These dipeptide nitriles were almost pure chromatographycally. In the case of DL-alanyl-DL- $\alpha$ -aminopropionitrile, the latter peak is smaller than the preceding one. This may be attributed to the separation of the dipeptide nitrile diastereomers occurring during recrystallization. The above described method may be useful for the synthesis of a dipeptide nitrile, especially one which has an optically active  $\alpha$ -aminonitrile group as its C-terminal residue. REFERENCES 1) K. Kawashiro, H. Yoshida, and S. Morimoto, Chem. Lett., <u>1974</u>, 1. 2) K. Kawashiro, H. Yoshida, and S. Morimoto, unpublished data. 3) D. W. Woolley, J. W. B. Hershey, and H. A. Jodlowsky, J. Org. Chem., <u>28</u>, 2012(1963). 4) L. Zervas, D. Borovas, and E. Gazis, J. Amer. Chem. Soc., <u>85</u>, 3660(1963). 5) S. Blackburn and P.Tetley, Biochim. Biophys. Acta, <u>20</u>, 423(1956). K. Noda, H. Okai, T. Kato, and N. Izumiya, Bull. Chem. Soc. Japan, <u>41</u>, 401(1968). J. M. Manning and S. Moore, J. Biol. Chem., <u>243</u>, 559(1968). 6) B. Liberek, A. Nowicka, and J. Szrek, Rocz. Chem., <u>39</u>, 369(1965).

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