Die Makromolekulare Chemie **175**, *105–110 (1974)*

Faculty of Engineering, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka, Japan

Studies on Functional Micelles, 1

Preparation of Cyclic Dipeptides from Phenylalanine *S*-Dodecyl Ester

YASUZIRO KAWABATA and MASAYOSHI KINOSHITA

Dedicated to Professor Dr. ICHIRO SAKURADA on his 70th birthday

(Date of receipt, August 14, 1973)

SUMMARY:

The hydrochloride of phenylalanine *S*-dodecyl ester (**2b**) reacts in pyridine/water at room temperature to afford 3,6-dibenzyl-2,5-piperazinedione (**3**) in good yields. Phenylalanine *S*-ethyl ester hydrochloride (**2a**), however, gives only small yields of **3** under the same conditions. This effect is explained with the assumption of a matrix reaction on the micelle surface. In contrast to the *S*-ethyl ester the reaction of the *S*-dodecyl ester leads to a mixture of two isomeric dioxopiperazines.

ZUSAMMENFASSUNG:

Das Hydrochlorid des Phenylalanin-*S*-dodecylesters (**2b**) reagiert im Pyridin/Wasser bei Raumtemperatur unter Bildung von 3,6-Dibenzyl-2,5-piperazindion (**3**) in guten Ausbeuten. Phenylalanin-*S*-äthylester-hydrochlorid (**2a**) liefert dagegen nur geringe Ausbeuten an **3** unter denselben Bedingungen. Dieser Effekt wird mit der Annahme einer Matrix-Reaktion an der Mizellen-Oberfläche erklärt. Im Gegensatz zum *S*-Äthylester führt die Reaktion des *S*-Dodecylesters zu einem Gemisch von 2 isomeren Dioxopiperazinen.

Introduction

The polymerization of vinyl monomers under the influence of any added polymer, the matrix polymerization, has attracted the interest of polymer chemists because of its analogy to the replication of biopolymers in biological organs and the possibility to utilize it for the synthesis of regulated polymer chains[1]. Some nonbonded interactions between polymerizing monomers, *e.g.* the formation of micelles, liquid crystals, or complexes through the side groups of the monomer also afford some specific propagation circumstances and result in the acceleration of the rate, in the depression of side reactions and in the enhanced stereospecificity of the resulting polymer[2–4]. The acceleration of the polymerization and the formation of polymers with extremely high molar masses in the polymerization of *p*-methacryloyloxy benzoic acid in the presence of *p*-cetyloxy benzoic acid are typical examples of a micelle effect in the vinyl polymerization[5]. KÄMMERER *et al.*[6] extended the matrix polymerization to the condensation of glycine, bound to phenol formaldehyde polycondensate, and obtained a cyclic dimer of glycine, the "dioxopiperazine".

With regard to peptide syntheses KATCHALSKY[7] carried out the condensation of higher alkyl esters of α-amino acids in a monolayer on water and observed the formation of a condensate which showed a positive biuret reaction. On the other hand SCHWYZER[8] reported

the promoted deamination of α-amino acid thiolesters catalyzed by amines. Many works on deamination and successive peptide synthesis from α-amino acid esters from aminoalkyl mercaptans were reported by WIELAND *et al.*[9].

This paper reports on the peptide synthesis especially of cyclic dipeptides from the *S*-dodecyl ester of phenylalanine (**2b**) catalyzed by pyridine, and on the acceleration of the rate through the intermolecular interaction of the dodecyl group compared to the ethyl group in the phenylalanine ester. The results afford a simplified model of cyclic oligopeptide syntheses proposed by LIPMANN[10] and a possible substitution of the polyfunctional catalysis of enzymes by micelle formation and subsequent reactions.

Experimental Part

Melting points are uncorrected. Products were identified by their IR (JASCO IR-G spectrometer) spectra and by paperchromatography developed on Whatman No. 1 or 3 filter paper, besides mp and elemental analyses.

Phenylalanine S-alkyl esters:

a) *Hydrochloride of phenylalanyl chloride* (**1**): D,L-Phenylalanine (10 g; 0,66 mol) in acetyl chloride (200 cm^3; 2,8 mol) was chlorinated with phosphorus pentachloride (15 g; 0,07 mol) according to the method of FISCHER[11]. Yield: 10 g (75 %).

b) *Phenylalanine S-ethyl ester hydrochloride* (**2a**): It was prepared by a modified route of WIELAND *et al.*[12]. A mixture of the hydrochloride of phenylalanyl chloride (**1**) (10 g; 45 mmol) and ethanethiol (25 g; 0,4 mol) was left at room temp. for 5 days and then poured into diethyl ether. The crude precipitate was recrystallized twice from ethanol/diethyl ether (1:5).; Colorless crystals; mp 166–168°C. Yield: 8 g (75 %).

$C_{11}H_{16}ClNOS$ (245,6)	Calc.	C 53,77	H 6,52	N 5,70
	Found	C 53,65	H 6,33	N 5,41

c) *Phenylalanine S-dodecyl ester hydrochloride* (**2b**): A solution of **1** (8 g; 36 mmol) and dodecanethiol (30 cm^3; 0,12 mol) in benzene (30 cm^3) was stirred at room temp. for 5 days. The crude precipitate obtained by adding the reaction mixture to petroleum ether, was recrystallized from acetone and twice from chloroform/diethyl ether. Colorless crystals; mp 98–99°C. Yield: 7 g (53 %).

$C_{21}H_{36}ClNOS$ (385,5)	Calc.	C 65,37	H 9,34	N 3,63
	Found	C 65,32	H 9,48	N 3,70

3,6-Dibenzyl-2,5-piperazinedione (**3**):

a) The authentic sample of *racemic* **3** was prepared by refluxing a solution of phenylalanine (5 g; 30 mmol) in ethylene glycol (50 cm^3) for 1 h and the crude product was recrystallized from ethanol; mp 277°C (Lit.[14]: 280°C). Yield: 1,0 g (22 %).

$C_{18}H_{18}N_2O_2$ (294,3)	Calc.	C 73,47	H 6,12	N 9,52
	Found	C 73,14	H 6,11	N 9,16

b) The *meso* form of **3** was obtained by heating phenylalanine ethyl ester at 180°C for 24 h[13]; mp 296–298°C (Lit.[13]: 300°C). Yield: 38 %.

c) To a mixture of **2b** (2,4 g; 6,2 mmol) and water (64 cm^3), pyridine (1,6 cm^3; 18,7 mmol) was added. The resulting turbid solution was allowed to stand at room temp. for a few weeks. After one day a tucky glassy mass deposited on the glass surface and changed gradually to a hard solid. The flocky precipitate was collected, refluxed with diethyl ether to remove didodecyl disulfide, and then recrystallized from ethanol. Two products were separated:

1) An ethanol soluble fraction as white crystals; mp 276–278 °C; mixed mp with *racemic* **3** showed no depression. Identification with the authentic sample by comparison of the IR spectra.

2) An ethanol insoluble fraction as amorphous powder, soluble only in hot acetic acid; mp 300–302 °C.

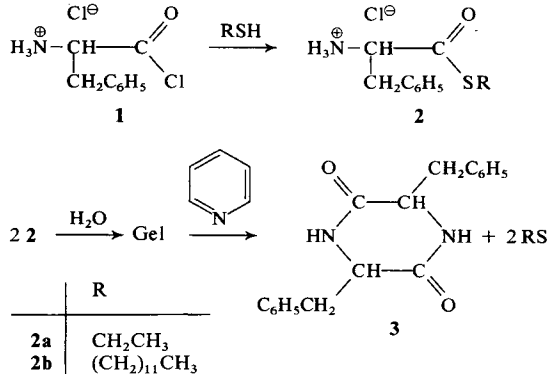
$C_{18}H_{18}N_2O_2$ (294,3) Calc. C 73,47 H 6,12 N 9,52
Found C 73,83 H 5,85 N 9,52

When the above reaction was carried out with 16,2 mmol of **2a** the collected crude precipitate was separated directly without recrystallization. Yields of both fractions are indicated in Fig. 2. A comparison of the yields with **2a** and **2b** is given in Tab. 1.

Other reagents: Phenylalanine dodecyl ester and phenylalanine ethyl ester were prepared according to the literature. Other reagents and solvents were commercially available and purified as usual.

Results and Discussion

Addition of pyridine to a mixture of **2b** and water resulted in the formation of *racemic* **3**.



In addition an appreciable amount of an ethanol insoluble amorphous powder was separated. This amorphous powder showed the same analytical data as compound **3**. Its IR spectrum, however, showed two splitted peaks at 1660 and 1675 cm^{-1} instead of a single peak at 1675 cm^{-1} but no absorption at 1550 cm^{-1} corresponding to an NH-absorption of a polypeptide. The compound was soluble only in hot acetic acid and has a mp 300–302 °C.

These data indicate that the compound is the *meso* form of **3**.

Yields of *meso* and racemic forms of **3** were plotted against the reaction time as shown in Fig. 1.

The ratio of isolated *racemic* and *meso* forms was about 3:1, although only one form was obtained in the synthesis of the authentic sample.

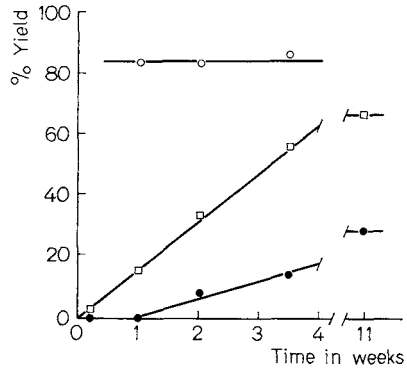


Fig. 1. Yield of 2,6-dibenzyl-2,5-piperazinedione (**3**) in the condensation reaction of phenylalanine *S*-dodecyl ester hydrochloride (**2b**) as a function of time. (Conditions: 1,2 g of **2b**; 32 cm^3 of water; 0,8 cm^3 of pyridine; room temp.). (□): meso **3**; (•): racemic **3**; (○): meso **3** + racemic **3** + recovered **2b**

From **2a** only a small amount of **3** was separated, and no formation of the amorphous powder was recognized, and in some cases unreacted **2a** was recovered.

Results of the condensation reaction are summarized in Tab. 1, together with some results obtained in the case of alanine *S*-alkyl esters.

These results show the great advantage of **2b** compared with **2a** in the preparation of **3** in pyridine/water. The yields of **3** are related to the isolated anhydride, the actual yields, therefore, must be higher. The reaction seems to be completed within several hours at 70 °C.

Syntheses of dioxopiperazines by polycondensation of the corresponding "free" alkyl esters of amino acids are reported in many works and used widely in practice. The appreciable difference in the yields obtained with the *S*-dodecyl ester **2b** and the *S*-ethyl ester **2a** may be explained in terms of the particular behaviour of the dodecyl residue, that is, of the long alkyl chain having an anionic part at its end. The significant differences in reactivity at fairly low reaction temperatures should be attributed to a higher reactivity of the –S–CO– bond towards the –N–H bond as shown in the literature. Reactions in which water is replaced by acetone, methanol, chloroform or benzene as solvent gave no peptide.

The experimental results may be explained assuming the participation of gel or micelle formation of the *S*-dodecyl ester. Experimental results with alanine *S*-alkyl esters also support this reaction pathway.

Tab. 1. Yields of 3,6-dibenzyl-2,5-piperazinedione (**3**) from phenylalanine *S*-dodecyl ester hydrochloride (**2b**) compared with those from phenylalanine *S*-ethyl ester hydrochloride (**2a**) at room temp.

No.	Amino acid *S*-alkylester hydrochloride	Mass in g	Pyridine V/cm³	Water V/cm³	Reaction time in days	Yield of compound **3** in mg	in %
1	**2b**	2,4	1,6	64	4	0,12	13
2	**2b**	1,2	0,8	32	30	0,30	66
3	**2b**	1,2	0	32	30	0	0[a]
4	**2b**	1,2	0,8	32	7,5 h (70°C)	0,27	66
5	**2b**	1,2	0,8	32	43	0,27	60
6	**2b**	1,2	0,8	32	38	0,33	72
7	**2b**	1,2	[b]	29	37	0,32	71
15	**2b**	1,2	[c]	32	8	0,11	23
16	**2b**	1,2	[d]	32	8	0,10	22
A-3	**A**[e]	0,98	0,8	32	15	0,12	52
A-4	**A**[e]	1,96	1,6	64	34	0,25	57
8	**2a**	0,76	0,8	32	8	0,061	13[a]
9	**2a**	0,76	0,8	32	38	0,041	9[a]
10	**2a**	0,76	0,8	32	7,5 h (70°C)	0,055	12
11	**2a**	0,76	0,8	32	23	0,084	19
12	**2a**	0,76	0	32	23	0	0
13	**2a**	0,76	[c]	32	23	0,33	72
14	**2a**	0,76	[c]	32	8	0,32	69
18	**2a**	0,76	[b]	32	37	0,26	58
19	**2a**	0,76	[d]	32	8	0,27	60
20	**B**[f]	0,71	0,8	32	7,0 h (100°C)	0	0[a, g]
A-16	**C**[h]	0,53	0,8	32	30	0	0[i]
A-19	**C**[h]	0,53	[c]	32	30	0	0[i]

[a] Unreacted starting material was recovered. [b] 3 cm³ of NaOH (c = 1 mol dm^{-3}). [c] 0,8 cm³ pyridine + 0,5 g imidazole. [d] 0,5 g imidazole. [e] **A** = alanine *S*-dodecyl ester. [f] **B** = phenylalanine ethyl ester. [g] Phenylalanine was isolated. [h] **C** = alanine *S*-ethyl ester. [i] Alanine was isolated.

[1] H. KÄMMERER, N. ÖNDER, Makromol. Chem. **111**, 67 (1968).
[2] V. A. KABANOV, O. V. KARGINA, V. A. PETROVSKAYA, Vysokomol. Soedin. **13**, 348 (1971).
[3] W. J. TOTH, A. V. TOBOLSKY, J. Polymer Sci. B **8**, 289 (1970).
[4] K. SHIMA, M. KINOSHITA, M. IMOTO, Makromol. Chem. **154**, 247 (1972).
[5] B. A. KRENTZEL, YU. B. AMERIK, Vysokomol. Soedin. **13**, 1358 (1971).
[6] V. BÖHMER, H. KÄMMERER, Makromol. Chem. **138**, 137 (1970).
[7] A. BANIEL, M. FRANKEL, I. FRIEDRICH, A. KATCHALSKY, J. Org. Chem. **13**, 791 (1948).
[8] R. SCHWYZER, Helv. Chim. Acta **36**, 414 (1953).
[9] T. WIELAND, H. BERNHARD, Liebigs Ann. Chem. **572**, 190 (1951).
[10] F. LIPMANN, Science **173**, 875 (1971).
[11] E. FISCHER, Ber. Deut. Chem. Ges. **38**, 2914 (1905).
[12] T. WIELAND, H. KOPPE, Liebigs Ann. Chem. **588**, 15 (1954).
[13] E. FISCHER, Ber. Deut. Chem. Ges. **34**, 433 (1901).
[14] E. SCHULZE, J. BARBIERI, Ber. Deut. Chem. Ges. **16**, 1711 (1883).