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Application of the Arndt-Eistert Synthesis to the Preparation of Polypeptides of β-Amino Acids

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The Wolff rearrangement of diazomethylphthalimidoalkyl ketones was performed in dioxane in the presence of esters of amino acids. The following polypeptides containing α- and β-amino acids, were thus prepared: β-alanyl-L-methionine (I), L-β-aminobutyryl-β-alanyl-L-methionine (II), L-β-aminobutyryl-glycine (III), L-β-aminobutyryl-L-methionine (IV), L-β-aminobutyryl-L-alanine (V) and β-alanyl-glycine (VI).

It has been shown by Balenović[1] that diazomethylphthalimidoalkyl ketones can be converted into the coresponding *β*-amino acids by the Arndt-Eistert synthesis. Following this method we have recently published a note in which a description for the preparation of dipeptides containing α- and *β*-amino acids was given[2]. The dipeptides were obtained as *N*-phthaloyl esters and in all but one case the optically inactive intermediates were used.

In this paper we describe the preparation of several optically active dipeptides and one tripeptide containing α- and β-amino acids. All these compounds were prepared following essentially the method set forth in the previous note[2]. The Wolff rearrangement of diazomethylphthalimidoalkyl ketones was performed in dioxane in the presence of silver oxide; the esters of amino acids were used as protonic reagents. The same method was extended to the preparation of tripeptides and L-β-aminobutyryl-β-alanyl-L-methionine (II) was

TABLE I

$$R \cdot NH \cdot \underset{\displaystyle R'}{\underset{|}{CH}} \cdot CH_2 \cdot CO \cdot NH \cdot \underset{\displaystyle R''}{\underset{|}{CH}} \cdot COOH$$

	R	R′	R″
I	H	H	$CH_2 \cdot CH_2 \cdot SCH_3$
II	$CH_3 \cdot CH(NH_2) \cdot CH_2 \cdot CO$.	CH_3	$CH_2 \cdot CH_2 \cdot SCH_3$
III	H	CH_3	H
IV	H	CH_3	$CH_2 \cdot CH_2 \cdot SCH_3$
V	H	CH_3	CH_3
VI	H	H	H

thus prepared from L-diazomethyl-α-phthalimidoethyl ketone and β-alanyl-L-methionine ethyl ester.

The esters of polypeptides were saponified with aqueous hydrochloric acid in acetone, following the method described by Sheehan and collaborators[3]. The phthaloyl group was removed by hydrazinolysis according to standard procedure[4].

The compounds described in this paper can be conveniently used for the preparation of various analogues of β-alethine, according to the method previously published[5].

EXPERIMENTAL*

L-*Methionine ethyl ester hydrochloride*

L-Methionine (5 g.) was suspended in 20 ml. of absolute ethanol saturated with dry hydrochloric acid and left for two days at room temperature. The solvent was evaporated in vacuo, hydrochloric acid removed by repeated evaporation with ethanol and the colourless oil dissolved in 5 ml. of ethanol and crystallized by addition of 20 ml. of ether. A yield of 5 g. (72%) of white needles was obtained, m. p. 79—81°. An analytical sample was recrystallized from a mixture of ethanol and ether until a melting point of 81—82° was obtained; $[\alpha]_D^{21}$ + 18.7° (c. 2.245% in ethanol).

Anal. 14.60 mg. subst.: 20.98 mg. CO_2, 9.70 mg. H_2O
$C_7H_{16}ClNO_2S$ (213.73) calc'd.: C 39.34; H 7.55%
found: C 39.21; H 7.43%

L-Methionine ethyl ester was obtained from the hydrochloride with dry ammonia in chloroform following the method of Viscontini[6]. The crude colourless

of a saturated sodium bicarbonate solution, treated with charcoal and precipitated with concentrated hydrochloric acid, and 3.5 g. of a yellow crystalline product obtained. The crude acid was recrystallized from 1.5 ml. of 96% ethanol to give 1.75 g. (31.2%) of white needles; m. p. 152—154°, $[\alpha]_D^{22}$ + 21.4° (c 2.3% in dioxane). A sample for analysis was crystallized twice from ethanol and melted at 155—155.5°, $[\alpha]_D^{22}$ + 21.2° (c 1.88% in dioxane).

Anal. 14.16 mg. subst.: 28.31 mg. CO_2, 6.30 m. H_2O
2.28 mg. subst.: 0.167 ml. N_2 (30°, 756 mm.)
$C_{16}H_{18}N_2O_5S$ (350.38) calc'd.: C 54.84; H 5.18; N 8.00%
found: C 54.56; H 4.97; N 8.20%

β-Alanyl-L-methionine (I)

Te crude acid Ib (1.3 g., 3.67 m*M*) was refluxed for two hours with 5 ml. of ethanol and 5 ml. of *M*-alcoholic hydrazine hydrate. After concentration under reduced presure, the residue was treated for ten minutes at 50° with 20 ml. of 5% hydrochloric acid, and allowed to cool for one hour to room temperature. The phthalylhydrazide was removed by filtration, and evaporation of the solvent yielded 0.9 g. of crude crystalline product. The crude hydrochloride was dissolved in 150 ml. of water (approximately 0.02 molar solution) and passed throught a 15 × 200 mm. column packed with Amberlit IR-4B acid absorbing resin. The column was washed with 150 ml. of water, and after evaporation of effluents under reduced pressure,

essentially the same way as described for the compound Ia. The crude product (2.3 g. of a yellow oil) was crystallized from 5 ml. of ethanol to give 0.3 g. (7%) of white crystals; m. p. 149—151°, $[\alpha]_D^{21}$ + 12.2° (c 1.3% in dioxane). A sample for analysis was crystallized from ethanol until a melting point of 154—155° was obtained, $[\alpha]_D^{21}$ + 21.6° (c 1.125% in dioxane).

stallized from a mixture of benzene and petroleum ether (2:1) to a melting point of 104—105°, $[\alpha]_D^{20}$ + 28.1° (c 1.67% in ethyl acetate).

Anal. 12.77 mg. subst.: 27.80 mg. CO_2, 5,85 mg. H_2O

at 70°. The crude product (1.4 g.) was crystallized from a mixture of 6 ml. of benzene and 10 ml. of petroleum ether to give 1 g. (48%) of white crystals; m. p. 124—126°, $[\alpha]_D^{22}$ + 13.9° (c 0.93% in ethyl acetate). A sample was crystallized from a mixture of benzene and petroleum ether (3 : 1) for analysis and had a melting point of 128—129°, $[\alpha]_D^{22}$ + 16.7° (c 1.62% in ethyl acetate).

Anal. 10.40 mg. subst.: 23.47 mg. CO_2, 5.90 mg. H_2O
3.38 mg. subst.: 0.255 ml. N_2 (29°, 757 mm.)
$C_{17}H_{20}N_2O_5$ (332.35) calc'd.: C 61.43; H 6.07; N 8.43%
found: C 61.58; H 6.34; N 8.49%

N-*Phthaloyl*-L-β-*aminobutyryl*-L-*alanine* (Vb)

The ester Va (0.7 g. 2.1 m*M*) was hydrolyzed with a mixture of 8.7 ml. of acetone, 4.2 ml. of water and 1.7 ml. of concentrated hydrochloric acid. Evaporation of acetone yielded 0.35 g. (55%) of a crystalline product, m. p. 170—171°, $[\alpha]_D^{20}$ + 48.6° (c 1.18% in dioxane). The melting point and the specific rotation did not change on further crystallization.

Anal. 18.06 mg. subst.: 39.19 mg. CO_2, 8.20 mg. H_2O
2.40 mg. subst.: 0.196 ml. N_2 (24°, 756 mm.)
$C_{15}H_{16}N_2O_5$ (304.29) calc'd.: C 59.20; H 5.30; N 9.21%
found: C 59.21; H 5.08; N 9.33%

L-β-*Aminobutyryl*-L-*alanine* (V)

The dipeptide V was prepared from 0.3 g. (1 m*M*) of the compound Vb, 2.3 ml. of ethanol and 2.3 ml. of *M*-hydrazine hydrate in ethanol. The free dipeptide obtained by means of Amberlit IR-4B was crystallized from a mixture of ethanol and water (1:4). A yield of 0.15 g. (88%) of the analytically pure product was obtained. M. p. 255—256°, $[\alpha]_D^{21}$ — 37.4° (c 1.26% in *N*-HCl).

Anal. 14.20 mg. subst.: 24.97 mg. CO_2, 9.98 mg. H_2O
2.44 mg. subst.: 0.338 ml. N_2 (23°, 761 mm.)
$C_7H_{14}N_2O_3$ (174.20) calc'd.: C 48.26; H 8.10; N 16.08%
found: C 47.09; H 7.86; N 15.99%

N-*Phthaloyl*-β-*alanyl-glycine* (VIa)

The compound VIa was prepared from 10 g. (34 m*M*) of N-phthaloyl-β-alanyl-glycine methyl ester[2] with 200 ml. of acetone, 160 ml. of water and 80 ml. of concentrated hydrochloric acid. Evaporation of acetone gave a yield of 4.75 g. (50%) of a crystalline product melting at 205—208°. A sample for the analysis was crystallized from ethanol until a melting point of 210—211.5°* was obtained.

Anal. 12.03 mg. subst.: 24.80 mg. CO_2, 4.68 mg. H_2O
$C_{13}H_{12}N_2O_5$ (276.24) calc'd.: C 56.52; H 4.38%
found: C 56.26; H 4.35%

β-*Alanyl-glycine* (VI)

The compound VIa (3 g., 10.8 m*M*) was hydrazinolyzed with 15 ml. of *M*-hydrazine hydrate in ethanol and 15 ml. of ethanol. A yield of 0.5 g. (32%) of needles was obtained. An analytical sample was crystallized from 70% ethanol to a melting point of 228—229°.*

Anal. 13.24 mg. subst.: 20.02 mg. CO_2, 7,95 mg. H_2O
1.37 mg. subst.: 0.235 ml. N_2 (27°, 752 mm.)
$C_5H_{10}N_2O_3$ (146.15) calc'd.: C 41.09; H 6.90; N 19.17%
found: C 41.26; H 6.71; N 19.31%

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* Reported[9] m. p. 212.5—213.5°.
* Reported[9] m. p. 229—230°.

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IZVOD

Pripravljanje polipeptida β-amino kiselina primjenom Arndt-Eistert-ove reakcije

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U prije objavljenoj publikaciji opisana je metoda pripravljanja dipeptida β-amino kiselina primjenom Arndt-Eistertove reakcije. Wolffovo pregrađivanje diazometil-α-ftalimidoalkil ketona provedeno je u dioksanu u nazočnosti estera amino kiselina. U ovoj publikaciji upotrebljena je ista reakcija za sintezu čitavog niza dipeptida i jednog tripeptida, koji sadrže optički aktivne α- i β-amino kiseline. Priređeni su slijedeći polipeptidi: β-alanil-L-metionin (I), iglice iz 50%-tnog etanola, t. t. 234°, $[\alpha]_D^{20}$ — 26.3° (c 2.34% u *N*-HCl); L-β-aminobutiril-β-alanil-L-metionin (II), kristalizirano iz smjese 90%-tnog etanola i etera (2:5), t. t. 227—229°, $[\alpha]_D^{20}$ + 4.02°±1.3° (c 1.12% u 10% HCl); L-β-aminobutiril-glicin (III), kristalizirano iz smjese etanola i vode (10:1), t. t. 236—237°, $[\alpha]_D^{21}$ + 12.4° (c 3.21% u vodi); L-β-aminobutiril-L-metionin (IV), pločice iz smjese etanola i vode (4:1), t. t. 250°, $[\alpha]_D^{20}$ — 16.8° (c 1.54% u *N*-HCl); L-β-aminobutiril-L-alanin (V), kristalizirano iz smjese etanola i vode (1:4), t. t. 255—256°, $[\alpha]_D^{21}$ — 37.4° (c 1.26% u *N*-HCl) i β-alanil-glicin (VI), iglice iz 70%-tnog etanola, t. t. 228—229°.

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