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Investigation of the Synthesis of Some Dehydroalanine Derivatives

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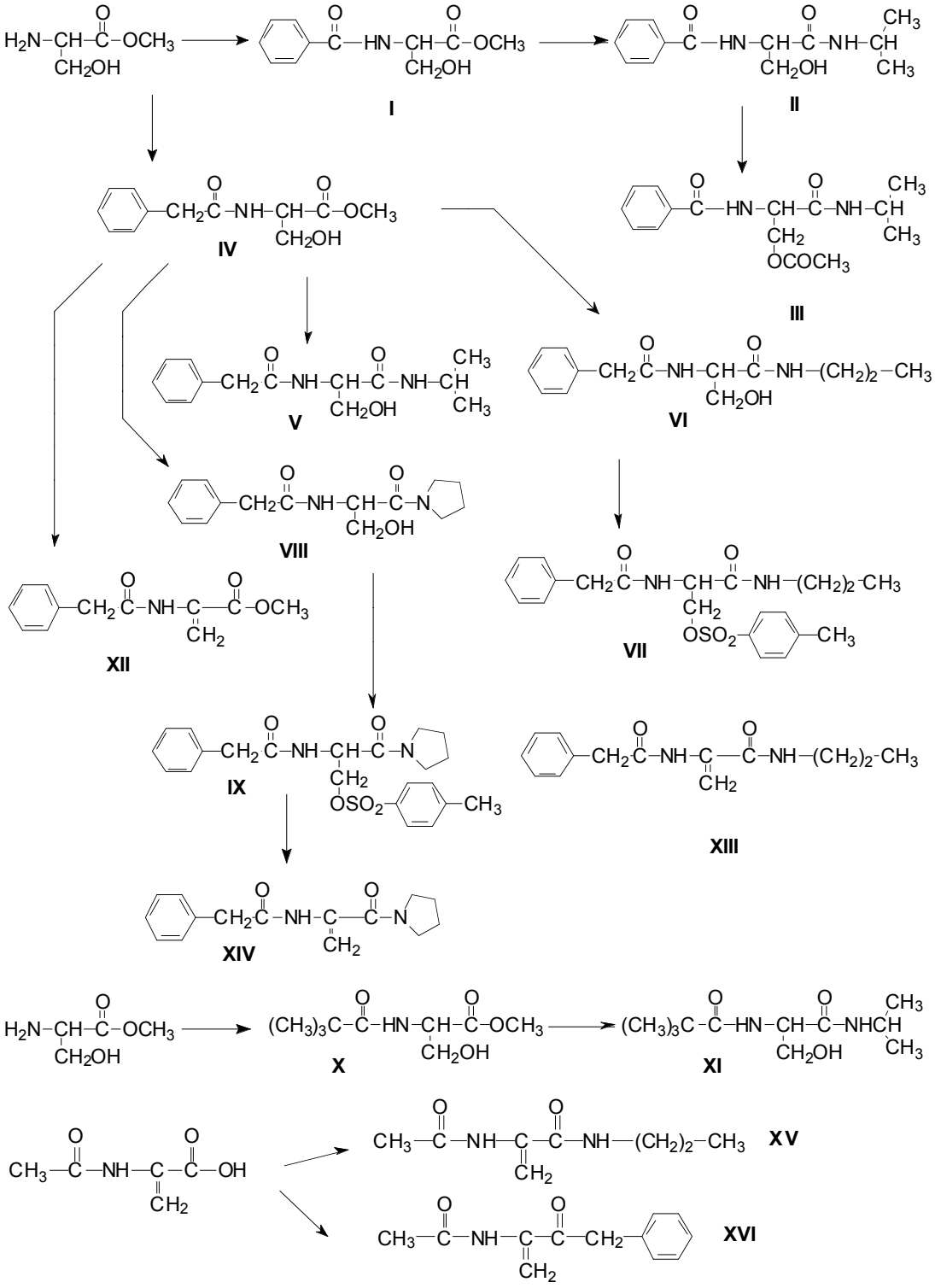
Dehydroamino acids represent an important class of compound as they are key intermediates in unusual amino acid and in the design of peptides and are constituents of a variety of naturally occurring antibiotic and phytotoxic peptides. In view of the importance of proteins and peptides containing hydroxyamino acids and the problems associated with their synthesis and dehydration, an investigation was performed on model compounds in order to examine the difficulties of the dehydration reactions. The preparation and properties of the model dehydroalanine derivatives have been studied.

Key Words: dehydroalanine, β-elimination, dehydration

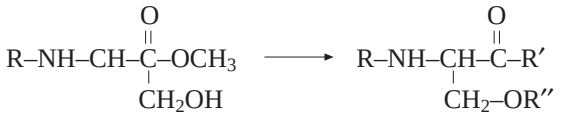
Introduction

Dehydroalanine is an α,β-unsaturated amino acid which plays a catalytic role in the active sites of some yeast[1] and bacterial[2] enzymes. It also occurs in a variety of peptide antibiotics of bacterial origin, including the "lantibiotics" nisin[3], subtilin[4], epidermin[5], and gallidermin[6], and more highly modified peptides such as thiostrepton, siomycin-A[7], nosiheptide[8], and berninamycin[9]. Novel active antibiotics[10] and immuno-adjuvants[11] have been synthesised by substituting dehydroalanine for other residues. Biosynthetically, the dehydroalanine in the lantibiotics originates from the dehydration of serine residues[12]. It has been postulated that dehydroamino acid plays an important role in giving the definite peptide conformation that is required for exhibition of biological activities[13,14]. Although several methods for synthesis of dehydroalanines have been reported15,16, there are still some problems such as unwanted side reactions or neighbouring effects of the protecting groups. At least ten dehydroamino acids have been found in nature while these and others may serve as precursors for other unusual, naturally occurring amino acids[17].

We report here some of the problems encountered during the synthesis of dehydroalanines derivatives. In order to examine the elimination reactions of serine derivatives, the amides and esters shown in Table 1 and Scheme 1 were synthesised.



Scheme 1. Reactions of N- and O-substituted serine derivatives



Scheme 2. Synthesis of substituted serine derivatives

Table 1. Yields and list of substituted serine derivatives

Compound	R	R‘	R‘’	Yield (%)
I	Bz	OCH_3	H	33
II	Bz	NH-$CH(CH_3)_2$	H	40
III	Bz	NH-$CH(CH_3)_2$	$COCH_3$	44
IV	Z	OCH_3	H	18
V	Z	NH-$CH(CH_3)_2$	H	38
VI	Z	NH-$(CH_2)_2CH_3$	H	72
VII	Z	NH-$(CH_2)_2CH_3$	$SO_2C_6H_4NH_2$	60
VIII	Z	NH-C_4H_8	H	59
IX	Z	NH-C_4H_8	$SO_2C_6H_4NH_2$	59
X	$(CH_3)_3$C-CO	OCH_3	H	49
XI	$(CH_3)_3$C-CO	NH-$CH(CH_3)_2$	H	66

N-Acyl and urethane protected serine amides were studied to mimic serine in a peptide chain, and were compared with esters which underwent elimination more readily. Preparation of the dehydroalanine derivatives shown in Table 2 was not straightforward. A number of methods were used including dehydration of serine derivatives and substitution of the serine OH followed by an elimination reaction to form the alkene derivative.

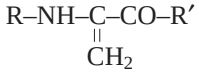


Table 2. N- and O-disubstituted dehydroalanine derivatives

Compound	R‘	R‘’	Yield (%)
XII	Z	OCH_3	59
XIII	Z	NH-$(CH_2)_2CH_3$	70
XIV	Z	NH-C_4H_8	42
XV	Ac	NH-$(CH_2)_2CH_3$	42
XVI	Ac	NH-$CH_2C_6H_4$	54

Experimental

All the chemicals were purchased from Aldrich Chemical Co. All melting points were determined using a digital melting point apparatus (Electrothermal IA9000 series) and were uncorrected. ^{1}H (400 MHz) and ^{13}C (62.9 MHz) NMR spectra were recorded on a Bruker AC 400 spectrometer. Tetramethylsilane was used as a reference. Unless otherwise stated, the deuteriated solvent was $CDCl_3$ and all the chemical shifts were measured from $SiMe_4$. J values are given in Hz. The low resolution mass spectra (EI/CI) were obtained with a Fisons VG Quattro II with NH_3 as reagent gas for CI. Accurate mass measurements were recorded on a VG ZAB E mass spectrometer. Column chromatography was carried out with silica gel 60 (230-400 Mesh). All the evaporations were made under reduced pressure. For the synthesis of N-protected serine

derivatives, Bergmann's general procedure[18] was employed. All the esterification reactions were carried out in dry methanol using thionyl chloride. Satisfactory yields were obtained at all stages and no special difficulties were encountered. The method of Harada and Tagasaki described[19] was used[20] to synthesise N-acetyldehydroalanine isopropyl amide **XV** (42%, mp 117.5-119.5°C) and N-acetyldehydroalanine benzyl amide **XVI** (54%, mp 123-124°C). The abbreviations used are as follows: DCCI (1,3-dicyclohexyl carbodiimide), DCU (1,3-dicyclohexylurea), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene), DEAD (diethyl azodicarboxylate), HOBt (1-hydroxybenzotriazole), Tosyl chloride (TsCl, p-toluene sulfonyl chloride), Bz (benzoyl) and Z (benzyloxycarbonyl).

N-Benzoyl-DL-serine isopropyl amide II

A solution of N-benzoyl-DL-serine methyl ester[21] **I** (0.46g) in isopropyl amine (5ml) was placed in a Teflon vessel which was sealed in a stainless steel container and heated in a Berghof block at 38-40°C for 3 days. Then the mixture was evaporated to dryness. The crude product was purified by column chromatography using ethyl acetate-petroleum spirit to give the pure amide **II** (0.2g, 40%), m.p. 147-149°C; δ_H (d_4-MeOH) 1.16, 1.17 (2×3H, 2d, J 6.6, CH(C<u>H</u>$_3$)$_2$), 3.85 (2H, d, J 5.5, CH_2), 4.00 (1H, q, CH(C<u>H</u>$_3$)$_2$), 4.58 (1H, t, J 5.5, CH-CO), 7.46 (2H, m, ArH), 7.54 (1H, m, ArH), (7.88 (2H,m, ArH); δ_C 22.60 [$(CH_3)_2$], 42.72 (CH(C<u>H</u>$_3$)$_2$), 57.21 (CH-CO), 63.27 (CH_2), 128.44, 129.52 and 132.87 (Ar-C), 135.09 (quarternary-C1), 169.87 (CH-C=O), 171.28 (Ph-C=O); (CI) m/z 251 (M+1), 233 [(M+1)-H_2O], 192 (M-NHCH$(CH_3)_2$); (EI) m/z 164 (5%, M-C_3H_7NHCO), 147 (25%, M+1-C_3H_7NHCO-H_2O), 105 (100%, Ph-CO), 77 (21%, C_6H_5); accurate mass (M+1) calculated for $C_{13}H_{19}N_2O_3$ 251.1396; found, 251.1391.

N-Benzoyl-O-acetyl-DL-serine isopropyl amide III

The method described by Srinivasan[22] was used. N-benzoyl-DL-serine isopropyl amide **II** (0.66g) was dissolved in dry pyridine (4ml) under N_2. The solution was cooled in an ice bath and acetic anhydride (5.38g) was added via syringe. The reaction mixture was stirred at room temperature for 1.5 days. Water (10ml) was added to the mixture and stirred for 15 min. The mixture was then extracted with CH_2Cl_2. The organic layer was washed with dilute HCl, saturated $NaHCO_3$, and water, dried and filtered, and the solvent was removed. The crude product was recrystallised twice from chloroform-petroleum spirit. The yield was 44%, m.p 154-155°C; δ_H 1.17, 1.18 (2×3H, 2d, J 6.6, CH(C<u>H</u>$_3$)$_2$), 2.09 (3H, s, CO-CH_3), 4.09 (1H, m, CH-(C<u>H</u>$_3$)$_2$), 4.36 (1H, dd, J 5.2, 11.5, 1H of CH_2), 4.55 (1H, dd, J 6.4, 11.5, 1H of CH_2), 4.85 (1H, dt, CH-CO), 6.23 (1H, d, J 6.5, NH), 7.18 (1H, bd, J 6.6, NH), 7.47 (2H, m, ArH), 7.54 (1H, m, ArH), 7.823 (1H, d, J 8.5, ArH), 7.826 (1H, d, J 8.1, ArH); (CI) m/z 293 (M+1), 234 (M-NHCH$(CH_3)_2$), 233 (M-$OCOCH_3$), 105 (Ph-CO), 77 (C_6H_5).

N-Benzyloxycarbonyl-DL-serine isopropyl amide V

A solution of N-Z-DL-serine methyl ester[21] **IV** (0.6g) in isopropylamine (10ml) was heated at 38-40°C for 3 days as described for the synthesis of compound **II**. The excess isopropylamine was evaporated. The crude product was crystallised from CH_2Cl_2-petroleum spirit to give fine white crystals (0.25g, 38 %, m.p. 132-134°C) of the product **V**; δ_H (d_6-DMSO) 1.02, 1.04 (2×3H, 2d, J 6.5 CH(C<u>H</u>$_3$)$_2$), 3.53 (2H, m, C<u>H</u>$_2$OH)), 3.81 (1H, m, CH(C<u>H</u>$_3$)$_2$), 3.98 (1H, m, CH), 4.82 (1H, t, J 5.7, CH_2O<u>H</u>), 5.03 (2H, s, CH_2-Ph), 7.10 (1H, d, J 8.5, NH), 7.36 (5H, m, ArH), 7.69 (1H, d, J 7.6, NH); (EI) m/z 281 (65%, M+1), 190 [100%, (M+1)-CH_2-Ph], 173 (17%, M-OCH_2Ph), 147 (11%, 190-C_3H_7), 108 (10%, $PhCH_2OH$), 91 (5%, C_7H_7); accurate mass (M+1) calculated for $C_{14}H_{21}N_2O_4$ 281.1528; found 281.1501.

N-Benzyloxycarbonyl-DL-serine propyl amide VI

A solution of N-Z-DL-serine methyl ester **IV** (0.8g) in propylamine (5ml) was stirred at room temperature for 24h as described for the preparation of compound **II**. The excess propyl amine was evaporated. The crude product was recrystallised twice from CH_2Cl_2 to give the crystalline compound VI in 95% yield (0.84g, m.p. 111°C). No NMR and mass spectral data were reported in the literature[23]. δ_H (d_4-MeOH) 0.90 (3H, t, C<u>H</u>$_3$-CH$_2$), 1.51 (2H, sextet, J 7.4, C<u>H</u>$_2$-CH$_3$), 3.14 (2H, t, C<u>H</u>$_2$-CH$_2$-CH$_3$), 3.73 (2H, d, J 5.3, C<u>H</u>$_2$OH), 4.15 (1H, t, J 5.3, CH), 5.09 (2H, s, CH$_2$-Ph), 7.32 (5H, m, Ar-H), δ_C : 11.73 (CH$_3$), 23.51 (<u>C</u>H$_2$-CH$_3$), 42.21 (<u>C</u>H$_2$-CH$_2$-CH$_3$), 58.45 (CH), 63.24 (<u>C</u>H$_2$-OH), 67.80 (<u>C</u>H$_2$-Ph), 128.92, 129.03 and 129.43 (ArCH), 137.92 (quarternary-C), 158.22 (C=O), 172.64 (C=O); (CI) m/z 281 (100%, M+1), 237 (3%, M-C_3H_7), 190 [31%, (M+1)-CH_2Ph], 173 (5%, M-OCH_2Ph), 147 (12%, 190-C_3H_7), 108 (8%, $PhCH_2OH$), 91 (3%, C_7H_7); accurate mass (M+1) calculated for $C_{14}H_{21}N_2O_4$ 281.1501, found, 281.1496

N-Benzyloxycarbonyl-O-tosyl-DL-serine propyl amide VII

The method described by Photaki[24] was used. A solution of N-Z-DL-serine propyl amide VI (1g) in dry pyridine was cooled at -10°C, and then TsCl (2.38g) in dry pyridine (5ml) was added dropwise to the reaction mixture under N_2. The reaction mixture was stirred first at -10°C for an hour and then at room temperature. The excess TsCl was destroyed by addition of a few drops of water. After the addition of water (15ml) the mixture was extracted with CH_2Cl_2 which was washed with 5% HCl, saturated $NaHCO_3$ and water. The organic layer was dried and evaporated to dryness. The product was obtained as a sticky yellow solid (0.2g) after purification by column chromatography (EtOAc-petroleum spirit). The yield was 17% (Lit[25], m.p. 91°C); δ_H 0.85 (3H, t, J 7.3, CH$_2$-C<u>H</u>$_3$), 1.44 (2H, sextet, CH$_2$-C<u>H</u>$_3$), 2.41 (3H, s, CH$_3$), 3.13 (2H, q, J 6.7, C<u>H</u>$_2$-CH$_2$-CH$_3$), 4.17 (1H, dd, J 5.1, 10.1, 1H of CH$_2$), 4.32 (1H, dd, J 5.1, 10.1, 1H of CH$_2$), 4.49 (1H, dt, CH), 5.96 (1H, d, J 8.1 NH), 6.59 (1H, bt, NH), 7.32 (7H, m, ArH), 7.73 (2H, d, J 8.2, H-2,6 in tolyl group); δ_C 11.24 (CH$_2$-<u>C</u>H$_3$), 21.66 (Ar-CH$_3$), 22.49 (<u>C</u>H$_2$-CH$_3$), 41.44 (<u>C</u>H$_2$-CH$_2$-CH$_3$), 53.76 (CH), 67.35 (CH$_2$-O), 69.14 (Ph-CH$_2$), 127.98-130.00 (ArCH), 132.02, 145.32 (23 quarternary-C), 156.03, 167.70 (2 C=O); (CI) m/z 435 (5%, M+1), 299 (20%, M-CO_2CH_2Ph), 280 [100%, (M+1)-$SO_2C_7H_7$], 263 [14%, (M+1)-$C_7H_7SO_3H$], 173 (10%, 280-OCH_2Ph), 108 (32%, $PhCH_2OH$), 91 (11%, C_7H_7); accurate mass (M+1) calculated for $C_{21}H_{27}N_2O6S$ 435.159, found, 435.1583

N-Benzyloxycarbonyl-DL-serine pyrrolidine amide VIII

A solution of N-Z-DL-serine methyl ester **IV** (1.19g) in dry pyrrolidine (4.5ml) was stirred at 55°C for one day. The excess pyrrolidine was evaporated to dryness. The crude product was crystallised from CH_2Cl_2-Et_2O twice to give a white crystalline compound **VIII** (0.81g) in 59% yield (m.p. 117-120°C) ; δ_H 1.88 and 1.97 (4H, 2m, N(C<u>H</u>$_2$-CH$_2$)$_2$), 2.60 (1H, bs, OH), 3.49 (3H, m, 3H of N(C<u>H</u>$_2$-CH$_2$)$_2$), 3.67 (1H, m, 1H of N(C<u>H</u>$_2$-CH$_2$)$_2$), 3.78 (1H, dd, J 4.2, 11.4, 1H of CH$_2$), 3.86 (1H, dd, J 4.0, 11.4, 1H of CH$_2$), 4.56 (1H, m, CH), 5.11 (2H, s, Ph-CH$_2$), 5.97 (1H, d, NH), 7.35 (5H, m, ArH); δ_C 24.07 and 25.96 (N(CH$_2$-<u>C</u>H$_2$)$_2$), 46.22 and 46.80 (N(<u>C</u>H$_2$CH$_2$)$_2$), 53.84 (CH), 63.37 (<u>C</u>H$_2$-OH), 67.07 (Ph-<u>C</u>H$_2$), 128.03, 128.16 and 128.51 (ArCH), 136.19 (quarternary-C), 156.40 (Z C=O),169.26 (amide C=O); (CI) m/z 293 (100%, M+1), 275 [3%, (M+1)-H_2O), 202 [88%, (M+1)-CH_2Ph], 185 (45%, M-OCH_2Ph), 108 (16%, $PhCH_2OH$), 72 (30%, $C_4H_{10}N$); accurate mass (M+1) calculated for C15H_20N_2O4 293.1501, found, 293.1501.

N-Benzyloxycarbonyl-O-tosyl-DL-serine pyrrolidine amide IX

The same reaction procedure was carried out as described for the synthesis of compound **VII** using N-Z-DL-serine pyrrolidine amide **VIII** (0.090g), TsCl (0.17g) in dry pyridine (1.5ml). The crude product was purified by crystallisation from CH_2Cl_2-Et_2O to give compound (in 59 % yield) as a pale yellow solid,

m.p. 89-91°C; δ_H 1.91 (4H, m, N(CH_2-C<u>H</u>$_2$)$_2$), 2.43 (3H, s, CH_3), 3.49 (4H, m, N(CH_2-CH_2)$_2$), 4.15 (2H, m, CH-C<u>H</u>$_2$-O), 4.78 (1H, m, CH), 5.06 (2H, s, Ph-CH_2), 5.61 (1H, d, J 7.5, NH), 7.33 (7H, m, ArH), 7.75 (2H, d, J 8.1, ArH); accurate mass (M+1) calculated for $C_{22}H_{27}N_20_6S$ 447.1590; found, 447.1583

N-Trimethylacetyl-DL-serine methyl ester X

To a vigorously stirred solution of DL-serine methyl ester HCl (1.7g) in saturated $NaHCO_3$ (25ml), N-trimethylacetyl chloride (1.72g) was added dropwise. Then the reaction mixture was stirred vigorously for 1.5 days at room temperature. The reaction was followed by TLC (MeOH-EtOAc, 1:1 detection with 1% KMnO4 in 10% NaOH[26,27]). The reaction mixture was extracted with CH_2Cl_2 and the organic layer was washed with 5% HCl, saturated $NaHCO_3$ water and dried. The crude product was purified by a column chromatography from EtOAc-MeOH (1.09g, 49% yield); δ_H 1.24 (9H, s, $(CH_3)_3$), 2.05 (1H, s, CH_2O<u>H</u>), 3.79 (3H, s, OCH_3), 3.88 (1H, dd, J 3.5, 11.2, 1H of CH_2), 3.97 (1H, dd, J 4.1, 11.2, 1H of CH_2), 4.64 (1H, q, J 3.8, CH), 6.68 (1H, d, J 7.2, NH); (EI) m/z 204 (28%, M+1), 186 [9%, (M+1)-H_2O], 173 [4%, (M+1)-OCH_3), 144 (11%, M-CO_2CH_3), 126 (20%, 144-H_2O), 85 (53%, Me_3CCO), 57 (100%, Me_3C); accurate mass (M+1) calculated for $C_9H_{18}NO_4$ 204.124, found, 204.126.

N-Trimethylacetyl-DL-serine isopropyl amide XI

The reaction that was described for the synthesis compound **II** was used. A solution of N-trimethylacetyl-DL-serine methyl ester **X** (1g) in isopropylamine (6ml) was heated at 40°C for 5 days. The reaction was followed by TLC[26,27]. The excess isopropylamine was then evaporated. The crude product was purified by column chromatography (EtOAc-MeOH) to give the pure amide **XI** (0.75g, 66%, m.p. 128-130.5°C); δ_H 1.12 and 1.16 (6H, dd, $(CH_3)_2$), 1.23 (9H, s, $(CH_3)_3$), 3.58 (1H, dd, J 4.4, 11.6 1H of CH_2), 3.65 (1H, bs, OH), 4.02 (1H, m, C<u>H</u>$(CH_3)_2$), 4.13 (1H, dd, J 2.8, 11.6, 1H of CH_2), 4.32 (1H, sept, CH-CO), 6.84 (1H, bd, N<u>H</u>CH$(CH_3)_2$), 6.86 (1H, bd, (CH_3)3C-CON<u>H</u>); (EI) m/z 212 (29%, M-H_2O), 200 [3%, (M+1)-CH_2OH), 187 (2%, M-C_3H_7), 173 (4%, M-Me3C), 155 (28%, 212-Me3C), 144 (33%, M-C_3H_7NHCO), 127 [96%, (M+1)-H_2O- C_3H_7NHCO], 85 (98%, Me3CCO), 57 (100%, Me_3C); (CI) m/z 231 (M+1); accurate mass (M+1) calculated for $C11H_{23}N_2O_3$ 231.1709, found, 231.1630

N-Benzyloxycarbonyldehydroalanine methyl ester XII

This compound was prepared from N-Z-DL-serine methyl ester **IV** according to Wojciechowska et al.[28] in 59% yield ; δ_H 7.31 (6 H, m, ArH and NH), 6.25 (1 H, bs, 1H of C=CH_2), 5.78 (1 H, d, J 1.5, 1H of C=CH_2), 5.15 (2 H, s, C<u>H</u>$_2$Ph) and 3.80 (3 H, s, OCH_3)

N-benzyloxycarbonyldehydroalanine propyl amide XIII

The N-Z-O-Ts-DL-serine propyl amide **VII** (0.1316g) was dissolved in dry methanol (2ml) under N_2. Na (7.76mg) in dry methanol (0.1ml), added to the reaction mixture via syringe and stirred for 30 min. A few ml of water were added and the mixture was then extracted with CH_2Cl_2. The organic phase was dried and evaporated. The crude product was purified by column chromatography (EtOAc/petroleum spirit) to give a white crystalline compound **XIII**. The yield 50%, m.p. 66-68°C (Lit[13] 68°C). No NMR or mass spectral data were published; δ_H 0.94 (3H, t, J 7.4, C<u>H</u>$_3$-CH_2), 1.56 (2H, sex, J 7.4, C<u>H</u>$_2$-CH_3), 3.29 (2H, dq, J 6.1, 7.4, C<u>H</u>$_2$-CH_2-CH_3), 5.06 (1H, t, J 1.7, C=C<u>H</u>), 5.16 (2H, s, Ph-CH_2), 6.04 (1H, bs, =CH), 6.19 (1H, bs, NH), 7.35 (5H, m, ArH), 7.59 (1H, bs, NH); °C 11.31 (CH_3), 22.66 (CH_2-CH_3), 41.81 (<u>C</u>H_2-CH_2-CH_3), 66.88 (Ph-<u>C</u>H_2), 97.75 (=CH_2), 128.11, 128.29 and 128.58 (ArCH), 134.69 (C=), 135.94 (quarternary-C), 153.32 and 163.62 (2 C=O); (CI) m/z 263 (100%, M+1), 219 (12%, M-C_3H_7), 155 (14%, M-OCH_2Ph), 129

(8%, M-CO_2CH_2Ph), 108 (5%, $PhCH_2OH$), 91 (7%, C_7H_7)

N-Benzyloxycarbonyl-dehydroalanine pyrrolidine amide XIV

N-Z-O-tosyl-DL-serine pyrrolidine amide **IX** (38 mg) was dissolved in dried CH_2Cl_2 (1.5ml) and Na (2mg) in dry MeOH (0.5ml) was added via syringe under N_2. The solution was stirred at room temperature for 1.5 h. The reaction solution was then washed with water. The organic layer was dried and filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography using EtOAc-petroleum-spirit to give the alkene **XIV** as an oil (10mg, 42% yield); δ_H 1.89 (4H, m, $N(CH_2\text{-}C\underline{H}_2)_2$), 3.52 and 3.65 (4H, dd, $N(C\underline{H}_2\text{-}CH_2)_2$), 5.03 (1H, s, C=CH), 5.14 (2H, s, Ph-$C\underline{H}_2$), 6.05 (1H, bs, C=CH), 7.34 (6H, m, Ph and NH); °C 24.00 and 26.56 ($N(CH_2\text{-}\underline{C}H_2)_2$), 47.02 and 50.13 ($N(CH_2\text{-}CH_2)_2$), 66.83 (Ph-$\underline{C}H_2$), 101.84 ($=CH_2$), 128.12, 128.24 and 128.55 (ArCH), 135.15 (C=), 136.01 (quaternary C), 153.37 (O=C-N), 164.80 (O=C-O).

Results and Discussion

Schmidt et al.[26] reported that the elimination of water from β-hydroxy-α-amino acid derivatives is a particularly valuable method for alkene synthesis. DCCI is one of the most common reagents used for the β-elimination[29,30]. N-Benzoyl-DL-serine methyl ester **I** was treated with DCCI and CuCl in dry CH_2Cl_2 under N_2 at -5°C and then 40°C overnight. The product signals were detected on the NMR spectrum (alkene protons δ_H 6.00 and 6.80 in $CDCl_3$), but during the purification, decomposition occurred. The same reaction, performed on the corresponding isopropyl amide II in DMF, gave a similar result. Dehydration of some serine methyl esters was achieved in acceptable yields by Wojciechowska et al.[28] who used DEAD and triphenyl phosphine as reagents. An attempt to prepare N-benzoyldehydroalanine isopropyl amide from the serine isopropyl amide II under these conditions gave 2-phenyl-4-isopropylamido-1,3-oxazoline resulting from neigbouring group participation of the N-benzoyl group. This oxazoline, which was isolated with some of the by-product, 1,2-diethoxycarbonylhydrazine, was identified by comparison of the ^1H and ^{13}C NMR data with that for the closely related 2-phenyl-4-methoxycarbonyl-1,3-oxazoline[31].

One important and well-used approach involves the β-elimination reactions of serine and threonine derivatives containing suitable leaving groups[18]. To synthesise N-benzoyl-O-tosyl-serine isopropyl amide, the alcohol **II** was treated with tosyl chloride in dry pyridine[18,24,25] at -5°C. The analyses of the crude product by NMR showed that the expected tosylate derivative was the minor product. The crude product was found to be unstable. Nakagawa et al.[32] studied the tosylation of hydroxy amino acids and found that the O-tosylated products gradually decomposed, especially in solution. Schluter et al.[33] mentioned the same problem and reported that the O-tosylated serine derivatives cannot be stored under atmospheric humidity. Their attempts to synthesise N-acetyl-O-tosyl-DL-serine methyl ester using TsCl in dry pyridine failed at several temperatures and reaction concentrations. Since carboxylic esters are more stable than sulphonic esters, the lithium bromide/DBU-promoted elimination of acetic acid[34] was next tried. N-Benzoyl-O-acetyl-DL-serine isopropyl amide **III** was prepared in 44% yield according to the method of Anderson et al.[35] and reacted with lithium bromide and DBU in dry THF. However, no alkene was detected and the starting material was recovered. In earlier work[9,23], it was found that N-benzyloxycarbonyl serine derivatives did not form oxazolines by neighbouring-group reaction of the urethane carbonyl group. N-Benzyloxycarbonyldehydroalanine methyl ester **XII** was synthesised successfully from N-benzyloxycarbonyl serine methyl ester IV as described by Wojciechowska et al.[28]. The NMR analyses showed the expected alkene

signals (δ_H 5.78 and 6.25 in $CDCl_3$). Wojciechowska et al.[28] gave the same signals δ_H values as 5.6 and 6.1. The alkene was found to be unstable at room temperature and under atmospheric humidity. Alternative dehydration conditions involving 1.17 equivalent of DCCI and 0.4 equivalent of CuCl in dry CH_2Cl_2 gave the alkene **XII** but in unsatisfactory yield and contaminated with DCU. The by-product DCU[36], which is removed by filtration, is not entirely insoluble, particularly in the presence of other dissolved materials. The solubility of DCU was investigated by Tartar and Gesquiere[37] who suggested that urea derivatives such as DCU had some solubility in almost all organic solvents.

N-Benzyloxycarbonyl-DL-serine propyl amide **VI** and N-benzyloxycarbonyl-DL-serine pyrrolidine amide **VIII** were easily synthesised using N-benzyloxycarbonyl-DL-serine methyl ester **IV** and tosylated. The tosylated compounds **VII** and **IX** were found to be unstable at room temperature and were stored in a refrigerator under N_2. To synthesise the dehydro derivatives **XIII** and **XIV** via tosyl elimination, a reaction with CH_3ONa was carried out. The tosyl elimination was found to be the best way to synthesise the dehydro derivatives of these and related amides. To investigate the tosyl elimination from compounds **VII** and **IX** using a different reagent from CH_3ONa two small-scale reactions were carried out using DBU[20] in $CDCl_3$ and d_6-DMSO. Both reactions were followed by NMR and the expected alkene signals were detected 4 min after the reaction was started. Alkene formation was complete in 40-45 min. In contrast, the reaction of the N-acylated amide **XI** with 4 equivalents of TsCl in dry pyridine at room temperature gave a low yield (7%) of the tosylate, which decomposed upon attempted recrystallisation.

Dehydration attempts of the N-benzyloxycarbonyl-DL-serine pyrrolidine amide **VIII** were unsuccessful due to decomposition of the resulting product, although the expected alkene signals (δ_H, 5.01 and 6.02 in $CDCl_3$) were detected by NMR. The reaction with DCCI (1.3eq.) was carried out in dry DMF in the presence of freshly purified CuCl. Synthesis of N-trimethylacetyl dehydroalanine isopropyl amide using 1.3 equivalent of DCCI in dry CH_2Cl_2 in the presence of CuCl was unsuccessful. An alternative succesful route to amide derivatives of N-acyldehydroalanine involved the coupling of N-acyldehydroalanine with amines in the presence of DCCI and N-hydroxysuccinimide[19]. The isopropyl and benzyl amides of N-acetyldehydroalanine were thus prepared[20].

Syntheses of some protected dehydroalanine derivatives (such as N-Bz, N-Z and N-trimethylacetyl protected) by β-elimination reactions of serine derivatives[9,30] were problematical because the dehydroalanine products are labile and decompose during the isolation[38].

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