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Resolution of DL-2-aminosuberic acid via protease-catalyzed ester hydrolysis

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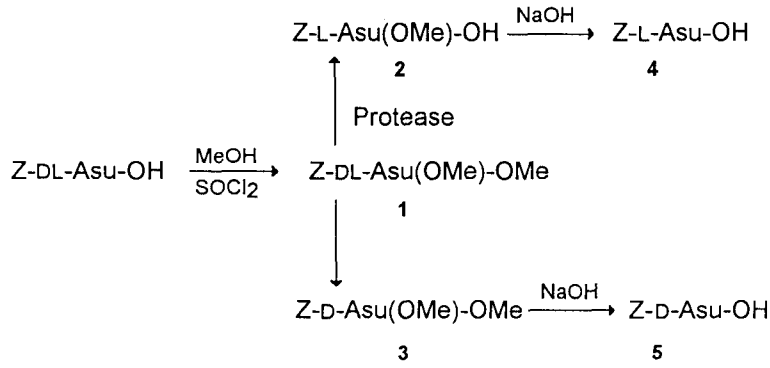
Summary. Papain-catalyzed regioselective cleavage of α-methyl ester in Z-DL-Asu(OMe)-OMe leads to Z-L-Asu(OMe)-OH and Z-D-Asu(OMe)-OMe. Subsequent saponifications yield Z-L-Asu-OH and Z-D-Asu-OH. The enzymatic α-ester hydrolysis was also achieved by subtilisin BPN′ in organic solvent with low water content.

Keywords: Amino acids – Aminosuberic acid – Enzymatic resolution – Papain – Subtilisin

Abbreviations: Asu: 2-aminosuberic acid, Z: benzyloxycarbonyl, OMe: methyl ester; DCHA: dicyclohexylamine

Introduction

2-Aminosuberic acid (Asu) is one of basic building blocks for the synthesis of peptide hormones analogs possesing an ethylene linkage instead of a disulfide bridge (the so-called carba analogs) (Jošt and Rudinger, 1967; Morikawa et al., 1976; Čeřovský et al., 1997). Resolution of its racemic form was achieved by fractional crystallization of benzyloxycarbonyl-DL-2-aminosuberic acid with D-tyrosine hydrazide or by enzymatic splitting of chloroacetyl-DL-2-aminosuberic acid with takadiastase (Hase et al., 1968). The papain-catalyzed synthesis of α-4-methylanilide of benzyloxycarbonyl-L-2-aminosuberic acid starting from Z-DL-Asu-OH and the following acidic hydrolysis, as performed in our laboratory, also gave L-Asu in satisfactory yield. However, it would be more advantageous to convert Z-L-Asu-4-methylanilide to Z-L-Asu-OH by a protease catalyzed 4-methylanilide hydrolysis. Unfortunately all attempts to use common proteases for this reaction failed. In the present study, as an alternative, we attempted to utilize the esterolytic activity of some



Scheme 1

proteases to hydrolyze the α-methyl ester functionality of Z-DL-Asu(OMe)-OMe (**1**) to get a mixture of Z-L-Asu(OMe)-OH (**2**) and Z-D-Asu(OMe)-OMe (**3**) as shown in Scheme 1.

Results and discussion

The diester **1** was prepared by esterification of Z-DL-Asu-OH with methanol-thionyl chloride. Papain and subtilisin were chosen as catalysts for the enzymatic ester hydrolysis. With papain, the reactions were performed in sodium acetate, pH 7.5, containing 20% dimethylformamide in order to increase solubility of the hydrophobic substrate **1**. We preferred to use only slightly alkaline medium in order to prevent the non-enzymatic ester hydrolysis. The reaction was monitored by HPLC until the ratio of compounds **2** and **3** reached 1:1. The products were then separated by extraction into organic (**3**) and aqueous (**2**) phases. The papain catalyzed hydrolysis of Z-DL-Asu(OMe)-OMe (**1**) proceeds, in the monophasic system, as expected. Somewhat surprisingly, utilization of a crude preparation of papain gave better results than application of the more expensive enzyme with higher purity. The papain-catalyzed hydrolysis was feasible also in a biphasic system of sodium acetate buffer and tert-butyl methyl ether. The subtilisin-catalyzed ester hydrolysis of **1** was carried out in acetonitrile containing 10% water with the equimolar amount of triethylamine (Čeřovský and Jakubke, 1996). However, the product **2** isolated from the latter reaction was contaminated by 10% of Z-Asu-OH. We explain this by inferior regioselectivity of subtilisin in organic solvents (Kitaguchi et al., 1991). Enantiomerically pure Z-L-Asu-OH (**4**) and Z-D-Asu-OH (**5**) were obtained by alkaline hydrolysis of **2** and **3** by 1M NaOH. Thus, the described simple three-step procedure utilizing an inexpensive enzyme (papain) provides a convenient preparative way to both enantiomers of 2-aminosuberic acid.

Materials and methods

DL-2-aminosuberic acid was synthesized in our laboratory by the acetamidomalonate procedure starting from caprolacton. Z-DL-Asu-OH was prepared by acylation of DL-Asu with N-(benzyloxycarbonyloxy) succinimide. The crude preparation of papain was purchased from Enzymase (Belgium), the purified papain from Fluka (Switzerland) and subtilisin BPN′ (Nagarse) from Serva (FRG). Melting points were determined on a Kofler block and are uncorrected. TLC was performed on Silufol plates (Kavalier, Czech Republic) coated with silica gel in four solvent systems. HPLC was carried out on a Thermo Separation Product instrument (USA) using a WP-300 Lichrosper C18 column (25 × 0.4 cm, 5 μm) from Merck (FRG). Various mixtures of methanol and 0.1% aqueous trifluoroacetic acid as eluent were used under isocratic conditions at a flow rate of 1 mL/min and a detection at 254 or 220 nm. Optical rotations were determined on Perkin-Elmer 141 MCA polarimeter.

*Z-DL-Asu(OMe)-OMe (**1**)*

Z-DL-Asu-OH (3.23 g, 10 mmmol) was esterified in cold dry methanol (50 mL) by adding thionyl chloride (1.6 mL) and stirring the solution overnight at room temperature. The solvent was evaporated, residue dissolved in tert-butyl methyl ether, washed with water, 0.5 M bicarbonate, water, dried and evaporated to give 3.5 g (99%) of the oily diester **1**. TLC, HPLC homogeneous, FAB-MS: 352.3.

*Z-L-Asu(OMe)-OH.DCHA (**2**) and Z-D-Asu(OMe)-OMe (**3**)*

a) With papain in monophasic system

Z-DL-Asu(OMe)-OMe (1.76 g, 5 mmol) was dissolved in a mixture of DMF (10 mL) and 0.2 M sodium acetate, pH 7.5 (40 mL). After the addition of Cys.HCl (15 mg) and crude papain (5 mg) the milky suspension was stirred overnight at room temperature. Two additional portions of Cys.HCl (15 mg) and the enzyme (5 mg) in one day intervals were necessary in order to complete the reaction. The mixture was acidified with 1 M HCl and the products (**2** and **3**) extracted with tert-butyl methyl ether. The product **2** was separated by extraction into 0.5 M $NaHCO_3$, then liberated by acidification (with cooling) and taken up in tert-butyl methyl ether. The solution was washed with water, dried and evaporated affording 0.77 g of oily Z-L-Asu(OMe)-OH (**2**) (91%). FAB-MS: 338.2. The TLC and HPLC homogeneous product **2** was obtained after crystallization from ether as the dicyclohexylammonium salt (0.89 g, 69%); m.p. 121°–123°C, [(Jošt and Rudinger, 1967) 123°–124°C], $[\alpha]_D$ + 7.15° (c 0.5, DMF). Elemental analysis: Calculated for $C_{29}H_{46}N_2O_6$ (518.7): 67.15% C, 8.94% H, 5.40% N; found: 67.09% C, 8.97% H, 5.40% N. The product **3** was isolated from the organic phase by washing with water, drying and evaporation. We obtained 0.82 g (93%) of oily TLC and HPLC homogeneous compound having the identical R_t on HPLC as **1**. FAB-MS: 352.1.

b) Preparation of **2** with papain in biphasic system

Z-DL-Asu(OMe)-OMe (355 mg, 1 mmol) was dissolved in tert-butyl methyl ether (10 mL) and 0.5 M sodium acetate pH 7.5 (10 mL). Cys.HCl (15 mg) and crude papain (5 mg) was added and the mixture was stirred overnight at room temperature. After the addition of the same amount of Cys.HCl and papain the mixture was stirred for another day. The compound **2** was isolated by the same procedure as described above giving 125 mg (74%) of oily Z-L-Asu(OMe)-OH which crystallized as Z-L-Asu(OMe)-OH.DCHA, 156 mg (60%).

c) Preparation of **2** with subtilisin in organic solvent

Z-DL-Asu(OMe)-OMe (350 mg, 1 mmol) was dissolved in a mixture of acetonitrile (9 mL), water (1 mL) and triethylamine (0.14 mL, 1 mmol). Subtilisin (10 mg) was

added and the mixture was stirred at room temperature. Two additional portions of enzymes were added in one day interval. After acidification of the mixture with 1M HCl the solvent was evaporated, the residue dissolved in tert-butyl methyl ether and the compound **2** was isolated by the same procedure as above. We obtained Z-L-Asu(OMe)-OH.DCHA, 180 mg (69%) contaminated with 10% of Z-Asu-OH.

Z-L-Asu-OH (4)

Z-L-Asu(OMe)-OH (300mg, oil) was hydrolyzed in methanol (2mL) by 1M NaOH (2mL) for 50min at room temperature. The mixture was acidified by 1M HCl, evaporated, the residue dissolved in ethylacetate, washed by 1M HCl and water, dried and evaporated. The product was crystallized from the mixture of ethylacetate and light petroleum to give 195mg (68%) of **4**, m.p. 119°-121°C, $[\alpha]_D$ −9.0° (c 1, DMF) [(Hase et al., 1968) 119°–121°C, $[\alpha]_D$ −9.1° (c 4, DMF)]. Elemental analysis: Calcd. for $C_{16}H_{21}N_1O_6$ (323.3): 59.43% C, 6.55% H, 4.33% N; found: 59.56% C, 6.62% H, 4.25% N. FAB-MS: 324.

Z-D-Asu-OH (5)

The compound **3** (400mg, oil) was hydrolyzed in methanol (3mL) by 2.2 equivalent of NaOH for 2.5 hours. The product was isolated by the same procedure as **4**. We obtained 255mg (69%) of 5, m.p. 119°–121°C, $[\alpha]_D$ +9.13° (c 1, DMF) [(Hase et al., 1968) 119°–121°C, $[\alpha]_D$ + 9.1° (c 4, DMF)] FAB-MS: 324.

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