

LIPIDIZATION OF SIMPLE AND DI-FUNCTIONAL AMINO ACIDS

(Penglipidan Asid Amino Ringkas dan Dwi-Fungsi)

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Abstract

This paper discuss the modification of azelaic acid into its applicable form by attachment of both its carboxyl sites to N-terminal of amino acid ethyl ester forming amide linkages in anhydrous medium. Acylation of glycine ethyl ester hydrochloride with azelaic acid dichloride was best conducted in a 100% anhydrous medium. L-amino acid ethyl ester bearing a primary hydroxyl group on its side chain gave mixtures of product and variation in composition depending on the mole ratio of reactants used. Reduction in purity was also observed for L-amino acid ethyl ester with primary –SH group on its side chain as compared to L-amino acid ethyl ester having –SCH₃ group on the L-amino acid side chain. The diamidoester of azelaic acid with L-alanine ethyl ester, L-valine ethyl ester, L-leucine ethyl ester and L-glutamic acid diethyl ester were in good yield when prepared through the modified Schotten-Baumann reaction conditions.

Keywords: Azelaic acid dichloride, L-amino acid ethyl ester, Schotten-Bauman condition, Glycine ethyl ester hydrochloride

Abstrak

Perbincangan membabitkan pengubahsuaian molekul dwi-asid karboksilik melalui lekatan pada kedua-dua kumpulan karboksil oleh terminal N-asid amino etil ester di dalam pelarut organik. Proses asilasi yang melibatkan tindakbalas di antara glisina etil ester hidroklorida dan asid azelik diklorida memberikan hasil dwi-amidoester tertinggi sekiranya dijalankan melalui proses asilasi di dalam 100% media organik kontang. L-asid amino yang mempunyai kumpulan sisi hidroksil primer memberi pelbagai campuran produk dan peratus komposisi yang berbeza bergantung pada nisbah reaktan yang digunakan. Penurunan dari segi ketulenan hasil diperolehi bagi L-asid amino yang mempunyai kumpulan sisi –SH berbanding dengan L-asid amino etil ester yang mempunyai kumpulan sisi –SCH₃. Dwi-amidoester asid azelik dari L-alanina etil ester, L-valina etil ester, L-leusina etil ester dan L-asid glutamik dietil ester adalah agak tinggi juga jika disediakan melalui proses asilasi melalui kaedah Schotten-Baumann yang diubahsuai.

Kata kunci: Asid azelik diklorida, L-asid amino etil ester, kaedah Schotten-Bauman, glisina etil ester hidroklorida

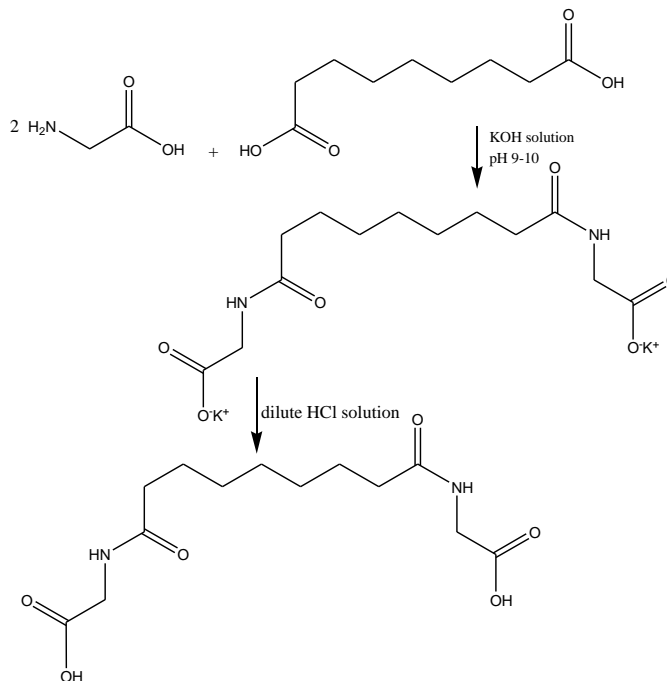
Introduction

Azelaic acid is a well known compound incorporated into topical cream medication formulation to treat patient with acne and skin diseases [1-5]. However, due to its crystalline form which melts at temperature as high as 103°C, incorporation of azelaic acid into cream formulation is, therefore, difficult at standard conditions. In addition, efficacy was only observed when 15% and above of azelaic acid was incorporated [6,7].

Maralmadi and Esposito (2002) [8] reported that modification of azelaic acid by attachment of glycine through amide linkages and converting one of the carboxylic acid group into potassium carboxylate has enhanced not only the technical property of azelaic acid, but also the functional performance of the resulting modified azelaic acid. Their studies showed that the modified azelaic acid, known as potassium azeloyl diglycinate, exhibited anti-acne, sebum regulatory and skin lightening properties on the treated skin.

The compound of interest has already been commercialized by Sinerga Italy and sold under the tradename Azeloglicina™. Attachment of glycine was performed through the conventional Schotten-Baumann reaction

condition i.e. by reacting 1 mol of azelaic acid dichloride with 2 mols of glycine in aqueous medium with regular pH control of 10.00 to 11.00. The di-potassium azeloyl diglycinate formed was then partially acidified with dilute hydrochloric acid solution to final pH of 5.5 to 6.0. The product was sold as 31% pale yellow solution. The reaction scheme is as illustrated in Scheme 1.



Scheme 1

In view of this valuable technical transformation of azelaic acid into its multifunctional tasks, we foresee the interest in producing this compound into its crystal form for ease of handling and transportation and to extend the method with other L-amino acid ethyl ester.

Materials and Methods

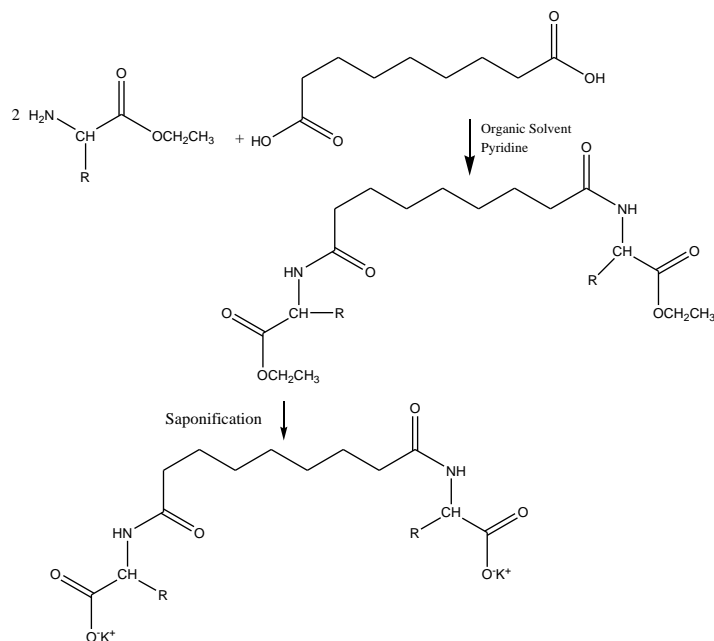
Glycine ethyl ester hydrochloride, L-serine ethyl ester hydrochloride and azeloyl dichloride with purity of above 98 % were purchased from Sigma-Aldrich Chemie GmbH (Switzerland). L-alanine and L-valine ethyl ester hydrochloride of purity above 99 % were purchased from Acros Organics, (USA). L-leucine ethyl ester hydrochloride and L-glutamic acid diethyl ester hydrochloride of GR Grade were purchased from TCI (Japan). L-cysteine ethyl ester hydrochloride of 98 % purity was purchased from Aldrich (Germany). All solvents used were of Analytical Grade.

Functional group characterization was conducted on FTIR instrumentation (Nicolet, MAGNA-IR 550 series II, Japan). The samples to be analyzed were first affixed on KBr pellet. Purity of the product isolated was determined using established HPLC methods [JASCO (Column: ODS-3(250mm x 4.6 mm) , Mobile phase: 35% acetonitrile and 65% H₂O, Detector: UV detector at 210 nm)].

¹H NMR-spectra of the isolated products was recorded on JEOL JNM-ECP400 FT NMR system (JEOL Ltd., Japan) with internal standard used was tetramethylsilane (TMS).

Reaction Between Azelaic Acid Dichloride and Glycine Ethyl Ester Hydrochloride in Anhydrous Medium

The method to produce diamidoester of azelaic acid with glycine ethyl ester was conducted according to process as described by Idris et al., (2009) [9]. The general reaction was as illustrated in Scheme 2.



Scheme 2

To evaluate the effect of amino acids with non polar side chain versus amino acids with polar side chain, the above reaction were also conducted using L-valine ethyl ester hydrochloride, L-alanine ethyl ester hydrochloride, L-leucine ethyl ester hydrochloride, L-glutamic acid diethyl ester hydrochloride, L-methionine ethyl ester hydrochloride, L-serine ethyl ester hydrochloride and L-cysteine ethyl ester hydrochloride as nucleophile. Their crude product mixtures were then analysed for their composition using HPLC system .

Saponification and Acidification of Diamidoester of Azelaic Acid with Glycine Ethyl Ester

The procedure to saponify the diamidoesters of azelaic acid with glycine ethyl ester was conducted according to the method described by Idris et al. (2009). The product was evaluated for its composition by HPLC, amide functionality and chemical formula confirmation by CHNO-S and ^1H and ^{13}C NMR.

Results and Discussion

Amidation in Anhydrous Medium with In-Situ Treatment of Amino Acid Ethyl Ester Hydrochloride

The reaction mixture was found to be homogeneous in aprotic solvent medium. As the reaction proceeded, slurry of salts crystals was formed. After recrystallization from isopropanol, the purity of the diamidoester was 97% (HPLC) with 50% recovery after first cycle of recrystallization from isopropanol.

Azeloyl diglycinate ethyl ester : Yield: 80.43%; Needle-like soft crystals flake. Melting point = 116.97°C. FTIR (KBr) (Figure 1a): 3318, 3085, 2985, 2930, 2850, 1751, 1644, 1544, 1377, 1350, 1190, 1022, 722 cm^{-1} . ^1H NMR (400Mhz, CDCl_3) (Figure 2): δ 1.26 (t, 6H), 1.30 (m, 6H), 1.61 (m, 4H), 2.21(t, 4H), 4.00(dd, 4H), 4.1 9(q, 4H), 6.21(s, 2H). Analysis: Calculated for $\text{C}_{17}\text{H}_{36}\text{N}_2\text{O}_6$: C, 56.97; H, 8.44; N, 7.82, Other, 26.78. Found: C, 57.52; H, 8.57; N, 7.90, Others, 23.48.

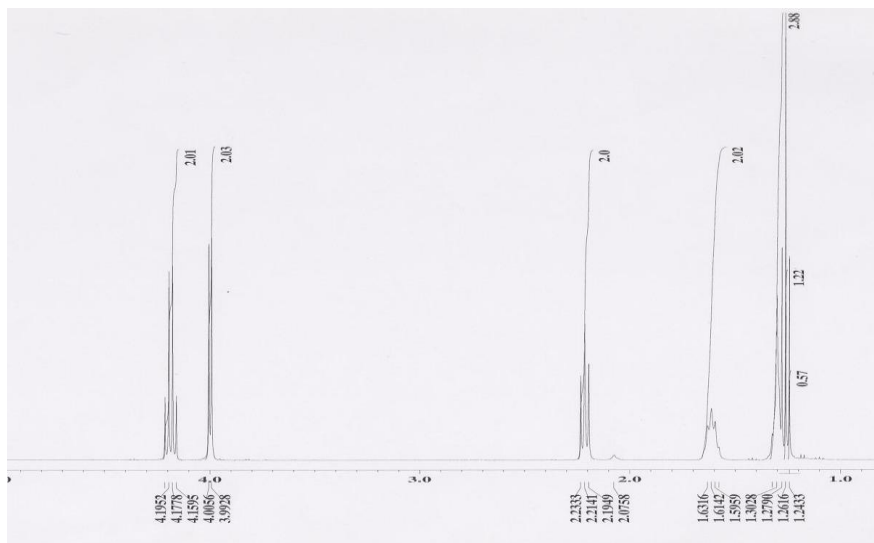
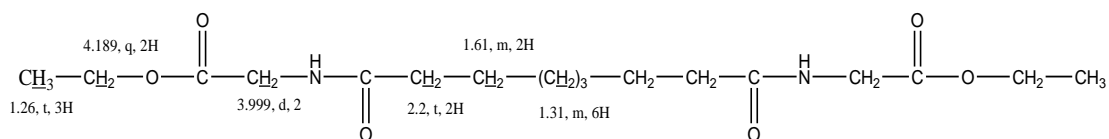


Figure 1. Overlaid FTIR spectrum of azeloyl diglycinate and its derivatives.

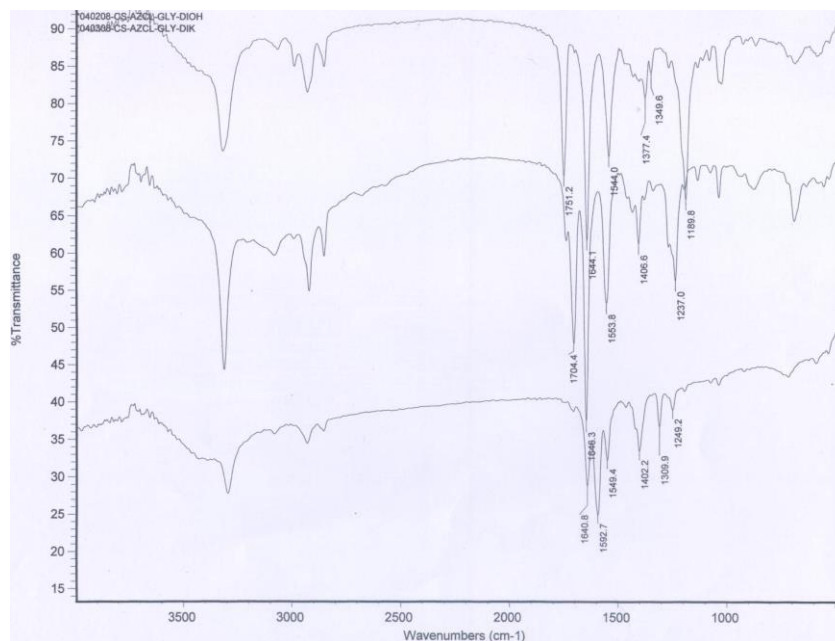


Figure 2. The ^1H NMR spectrum of azeloyl diglycinate ethyl ester with its predicted structural illustration above the spectrum

Saponification and Acidification

Diamidoester of azelaic acid with glycine ethyl ester was saponified through the usual saponification procedure to form its dipotassium carboxylate compound. Further derivatization through acidification in aqueous solution with dilute hydrochloric acid gave a product of diamido glycinate of azelaic acid. The following analysis results indicated that the amide linkages on both sides of azelaic acid were retained through the process.

Azeolyl diglycinate:Yield: white soapy granulated solid; Melting point= Nil; ash residue: 10.86%. FTIR (KBr)(Figure 1c): 3295, 3085 (weak), 2930, 2850, 1751(weak), 1641, 1593, 1549, 1402, 1310, 1190 (weak), 718, cm^{-1} . ^1H NMR (CDCl_3)(Figure 3): δ 1.28 (m, 6H), 1.56 (m, 4H), 2.25 (t, 4H), 3.70 (s, 4H).. Analysis: Calculated for $\text{C}_{13}\text{H}_{20}\text{K}_2\text{N}_2\text{O}_6$: C, 40.25; H, 5.33; N, 7.40; Others, 46.02. Found: C, 39.19; H, 5.69; N, 7.66; Others, 47.46.

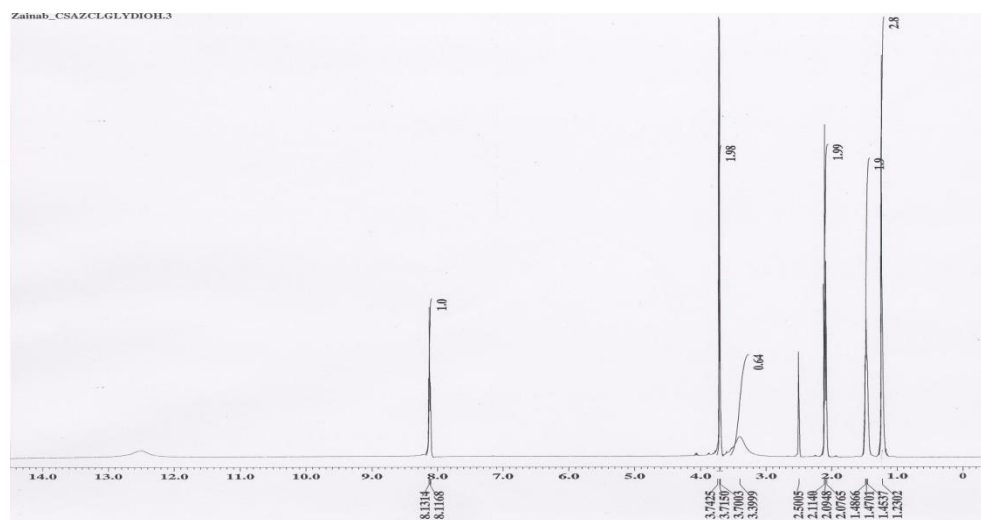
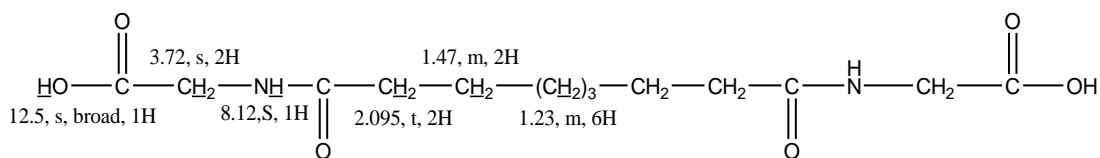


Figure 3. The ^1H NMR spectrum of Azeolyl diglycinate with its predicted structural illustration above the spectrum

Dipotassium azeolyl diglycinate:Yield: Fine white powder; melting point = 189.6°C. FTIR (KBr)(Figure 1b): 3295, 3066 (weak), 2990 (weak), 2930, 2850, 1751(weak), 1704, 1646, 1553, 1407, 1237, 1190 (weak), 688 (stronger) cm^{-1} . ^1H NMR ($\text{DMSO}_4\text{-D}_6$)(Figure 4): δ 1.23 (m, 6H), 1.47 (m, 4H), 2.09 (t, 4H), 3.40 (s, 2H), 3.72 (dd, 4H), 8.12 (t, 2H), 12.51 (s, 1H). Analysis: Calculated for $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_6$: C, 51.65; H, 7.33; N, 9.27; Others, 31.75. Found: C, 51.87; H, 7.38; N, 9.67; Others, 29.97

Effect of Non Polar Side Chain Versus Polar Side Chain Of Amino Acid Ethyl Ester

Based on the data in Table 1, it was interesting to note that among all the L-amino acid ethyl ester studied, L-serine ethyl ester possessing a primary alcohol side chain was found to be the most reactive to side chain reactions. With 2:1 mole ratio of L-serine ethyl ester to azeloyl dichloride, only 9.58 % of the organic soluble fraction was isolated

as crude product. The rest of the fraction was soluble in the aqueous phase and was discarded during the washing process. The major component in organic soluble fraction was 51% pure. On the other hand, reaction conducted with 1:1 mole ratio gave a higher crude yield but the purity of the major peak detected for 2:1 ratio was drastically reduced to 6.20 % and could have been converted to a less polar component which appears at the far end of the HPLC acquisition time. The side chain of neutral amino acid ethyl esters: L-valine ethyl ester, L-alanine ethyl ester, L-leucine ethyl ester and L-glutamic acid diethyl ester seemed to be the least affected as their purity was above 80 %. This was because the side chain of the first three L-amino acid ethyl esters was purely hydrocarbon, while that of L-glutamic acid diethyl ester, the carboxylic acid functionality on the side chain was protected by presence of an ethyl ester group.

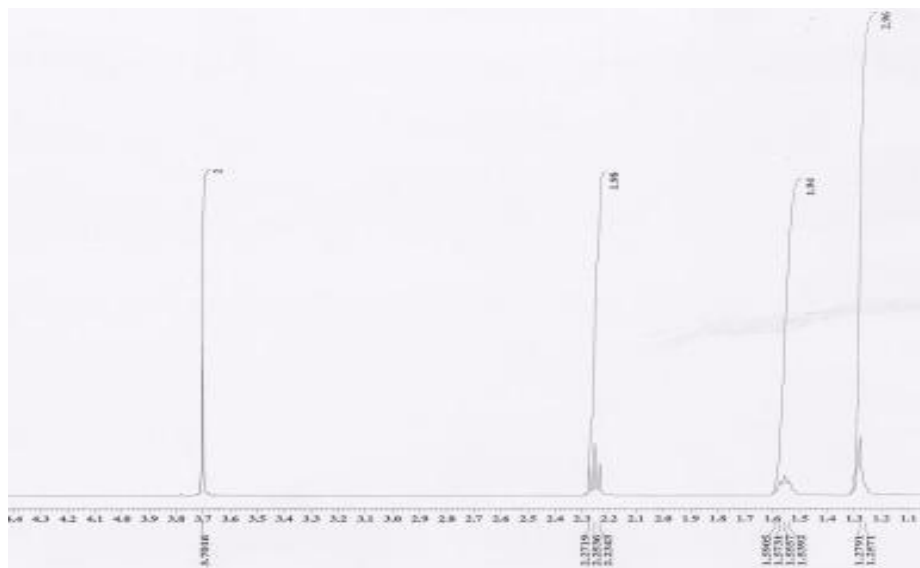
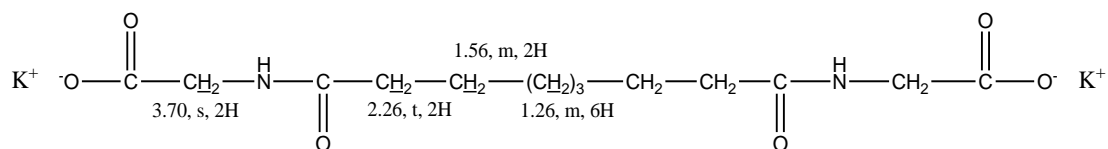


Figure 4. The ^1H NMR spectrum of dipotassium azeloyl diglycinate with its predicted structural illustration above the spectrum

Table 1. Characterization of reaction products for reaction between various L-amino acid ethyl ester hydrochloride and azeloyl dichloride

Types of L-amino acid ethyl ester hydrochloride	Crude yield ^a (%)	Purity ^b (HPLC) (%)
L-valine ethyl ester	76.38	81.76
L-alanine ethyl ester	87.01	86.74
L-leucine ethyl ester	83.64	82.20
L-serine ethyl ester (2:1) ^c	9.58	50.78*
L-serine ethyl ester (1:1) ^d	44.00	6.20*
L-cysteine ethyl ester	76.92	49.10*
L-methionine ethyl ester	75.53	70.56
L-glutamic acid diethyl ester	79.27	89.89

^aCrude yield was calculated based on the expected yield of diamidoester of respective amino acid ethyl ester.

^bThe % purity was based on the % area of the major peak appearing at the earliest retention time of HPLC chromatogram. ^cReaction between L-serine ethyl ester and azeloyl dichloride at 2:1 mole ratio. ^dReaction between L-serine ethyl ester and azeloyl dichloride at 1:1 mole ratio

Conclusion

The amidation of azeloyl dichloride and glycine ethyl ester in 100% anhydrous medium has provided a simpler method in producing diamidated azelaic acid with purity of above 97%. Organic base can be used to remove the hydrochloric acid molecules both from glycine ethyl ester hydrochloride and that resulting from the reaction itself. Unreacted organic base and the resulting hydrochloride salts were easily removed through subsequent washing with diluted HCl solution and followed by water. Aprotic solvent medium and its traces could be completely removed by vacuum evaporation followed by saponification in ethanolic potassium hydroxide and then acidification by dilute acid resulting in precipitation of precipitating the azeloyl diglycinate out from the aqueous media.

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