# DIAMIDATION OF AZELAIC ACID IN ANHYDROUS MEDIUM

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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 $–SCH_3$ group on the L-amino acid side chain. The specific rotation of all the azelaic acid diamidoester of L-amino acid ethyl esters studied were in the range of between $-17.48^o$ to $-44.10^o$ except for that of L-serine ethyl esters giving only $-3.81^o$ for 2:1 reactant ratio and $+6.92^o$ for 1:1 reactant ratio. 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.*

## INTRODUCTION

Azelaic acid is a well known compound that can be incorporated into topical cream medication formulation to treat patient with acne and skin diseases (Bojar et al., 1994, Thiboutot, 2002 and Akamatsu et al., 1991). However, due to its crystalline form which melts at temperature as high as 103$^{o}$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 (Webster, 2000, Hermanns, 2001).

Maralmadi and Esposito (2002) 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 at low dosage.

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 mol of glycine in aqueous medium with a 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.

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.

## EXPERIMENTAL APPROACH

Raw materials:

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 which was donated by JICA, Japan (Nicolet, MAGNA-IR 550 series II, Japan). The samples to be analysed

were first affixed on KBr pellet. Purity of the product isolated was determined using established HPLC methods.

$^{1}H$ and $^{13}C$ NMR-spectra of the isolated products were recorded on JEOL JNM-ECP400 FT NMR system (JEOL Ltd., Japan). The value for specific rotation of the reaction products of L-amino acid ethyl ester were determined on an automatic polarimeter ATAGO AP100 (Atago Co. Ltd, Japan) using a light source of $\lambda$ = 589 nm.

## METHODS

*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).

To evaluate the effect of neutral amino acids versus amino acids with active 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 according to the indicated conditions and specific rotation at $20^{o}C$ in absolute ethanol.

*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 $^{1}H$ and $^{13}C$ NMR.

## RESULTS AND DISCUSSIONS

*Amidation in anhydrous medium with in-situ treatment of amino acid ethyl ester hydrochloride.* The reaction mixture was found to be homogeneous in the aprotic solvent medium. As the reaction proceeds, 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.

Crude yield: 80.43%; Needle-like soft crystals flake, Mp$^{o}$C=116.97$^{o}$C. FTIR(KBr): 3318, 3085, 2985, 2930, 2850, 1751, 1644, 1544, 1377, 1350, 1190, 1022, 722 cm$^{-1}$.$^{1}$H NMR (400Mhz,$CDCl_3$):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). $^{13}$C NMR. (100Mhz, $CDCl_3$): δ 14.28 (2C), 25.53 (2C), 29.01(3C), 36.4(2C), 41.45 (2C), 61.6 (2C), 170.32 (2C), 173.48 (2C). Analysis: Calculated for $C_{17}H_{36}N_2O_6$: C, 56.97; H, 8.44; N, 7.82, Other, 26.78. Found: C, 57.52; H, 8.57; N, 10.43, Others, 23.48. The chemical structure of the compound was as shown in Figure 1.

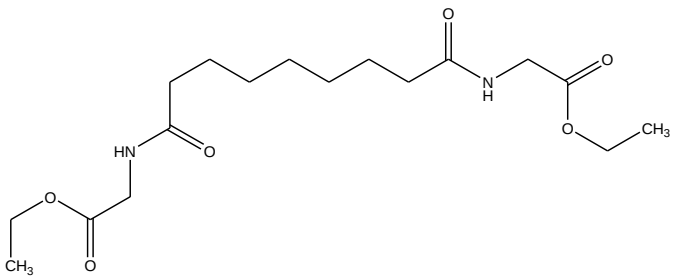


Figure 1: Chemical structure of the diamidated azelaic acid with glycine ethyl ester

*Saponification and acidification*: Diamidoester of azelaic acid with glycine ethyl ester was saponified through a usual saponification procedure to form its dipotassium carboxylate compound Further derivatization through acidification in aqueous solution with dilute HCl 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.

Physical Appearance: white soapy granulated solid; mp: Nil; ash residue: 10.86%. FTIR (KBr): 3295, 3085 (weak), 2930, 2850, 1751(weak), 1641, 1593, 1549, 1402, 1310, 1190 (weak), 718, cm$^{-1}$. $^{1}$H NMR ($CDCl_3$): δ=1.28 (m, 6H), 1.56 (m, 4H), 2.25 (t, 4H), 3.70 (s, 4H). $^{13}$C NMR ($CDCl_3$): δ=25.53(2C), 28.24 (2C), 28.19 (1C), 35.88 (2C); 43.41 (2C), 177.0 (2C), 177.23 (2C). Analysis: Calculated for $C_{13}H_{20}K_2N_2O_6$: C, 41.25; H,5.33 ; N, 7.40; Others, 46.02. Found: C, 39.19; H, 5.69; N, 7.66; Others, 47.46.

Physical appearance: Fine white powder; mp 189. 6$^{o}$C. FTIR (KBr): 3295, 3066 (weak), 2990 (weak), 2930, 2850, 1751(weak), 1704, 1646, 1553, 1407, 1237, 1190 (weak), 688 (stronger) cm$^{-1}$. $^{1}$H NMR ($DMSO_4$-$D_6$): 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). $^{13}$C NMR ($DMSO_4$-$D_6$): δ= 25.22 (2C), 28.25 (2C), 28.63 (1C), 40.50

(2C), 171.53 (2C), 172.67. Analysis: Calculated for $C_{13}H_{22}N_2O_6$: C, 51.65; H, 7.33; N,9.27; Others, 31.75. Found: C, 51.87; H, 7.38; N, 10.78; Others, 29.97

*Effect of neutral versus functional side chain of amino acid ethyl ester:* Based on the summarized data tabulated 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% purity. 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 may have been converted to a less polar component which appear 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 seems to be the least affected as their purity was above 80 %. This is 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.

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) (%)** | **Specific rotation at 20°C $[\alpha]_{20}$** |
|---|---|---|---|
| L-valine ethyl ester | 76.38 | 81.76 | -23.05° |
| L-alanine ethyl ester | 87.01 | 86.74 | -44.10 ° |
| L-leucine ethyl ester | 83.64 | 82.20 | -30.99 ° |
| L-serine ethyl ester (2:1)[c] | 9.58 | 50.78 | -3.81 ° |
| L-serine ethyl ester (1:1)[d] | 44.00 | 6.20 | +6.92 ° |
| L-cysteine ethyl ester | 76.92 | 49.10 | -24.5 ° |
| L-methionine ethyl ester | 75.53 | 70.56 | -22.96 ° |
| L-glutamic acid diethyl ester | 79.27 | 89.89 | -17.48 ° |

[a]Crude yield was calculated based on the expected yield of diamidoester of respective amino acid ethyl ester.

[b]The % purity was based on the % area of the major peak appearing at the earliest retention time of HPLC chromatogram.

[c]Reaction between L-serine ethyl ester and azeloyl dichloride at 2:1 mole ratio.

[d]Reaction between L-serine ethyl ester and azeloyl dichloride at 1:1 mole ratio.

## CONCLUSIONS

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 molecule both from glycine ethyl ester hydrochloride and that resulting from the reaction itself. Unreacted organic base and the resulting hydrochloride salts can then be easily removed through subsequent washing with diluted HCl solution and followed by water. Aprotic solvent medium and its traces could be completely removed through vacuum evaporation, saponification in ethanolic potassium hydroxide and acidification precipitating the azeloyl diglycinate out from the aqueous media.

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