Surface Active N-Acylglutamate: I. Preparation of Long Chain N-Acylglutamic Acid

MASAHIRO TAKEHARA, IPPEI YOSHIMURA, KOUICHI TAKIZAWA and **RYONOSUKE YOSHIDA,** The Central Research Laboratories of Ajinomoto Co., Inc., Suzuki-cho, Kawasaki, Japan

ABSTRACT

Long chain N-acylglutamic acid was prepared in a high yield by a reaction of glutamic acid with fatty acid chloride in a mixed solvent of water and a water miscible organic solvent such as acetone, methyl ethyl ketone, dioxane, tetrahydrofuran, *t*-butyl alcohol or cyclohexanone. In this reaction the composition of the mixed solvent influenced the yield of N-acylated glutamic acid and the best yield was obtained when the reaction was carried out in the mixed solvent comprising 30-60% v/v of the organic solvent. Long chain N-acylaspartic acid was also obtained in a high yield by the same method. As the other method to obtain N-lauroyl-DL-glutamic acid, it was examined that N-acyl-α-aminoglutarodinitrile which was obtained by a reaction of α-aminoglutarodinitrile with fatty acid chloride was hydrolyzed with an aqueous alkaline solution. The salts of long chain N-acylglutamic acid are known as the surface active agents that react mildly on the human skin.

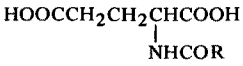
INTRODUCTION

The salts of long chain N-acylamino acids are generally known as the surface active agents having the long chain acyl radical as a lyophilic group and the salt of carboxylic acid as a hydrophilic group. It has been recognized that the salts of N-acylamino acids have generally a good solubility, a good detergency and a lime resistance. And moreover these compounds are mild and less irritating to skin or textiles and many of them are antimicrobial. Sodium N-acylsarcosinate is the best-known surfactant in this field and has been used as a dentifrice because of its anticaries action. The derivative obtained by acylating the hydrolyzed waste protein has been known as a less irritating and milder surfactant on the skin.

In the early research of long chain N-acylamino acids, Bondi (1) in 1909 prepared N-lauroylglycine and N-lauroylalanine and was followed by Abderhalden and Funk (2) and Izar (3). Karrer et al. (4) studied the formation of ethoxyoxazoles from the ethylesters of long chain N-acylamino acids. It was reported in early patents that the salt of N-acylaspartic acid was used as a wetting agent foı textiles (5) and that the salts of N-acylsarcosine, N-acylglycine and the like were suitable for detergents, textile cleaning agents, shampoos and dentifrices (6). Staudinger and Becker (7) examined the solubilities and viscosities of N-acylsarcosine and the like. Earlier researches mainly examined the enzymatic hydrolysis of long chain N-acylamino acids and only a few surface activities were investigated. Naudet (8) prepared N-acylserine and N-acylleucine to estimate both surface and interfacial tensions of the corresponding sodium salts. Tsubone (9) has investigated the surface activities of aqueous solutions of sodium salts of N-acylvaline and the like. Heitmann (10) examined the critical micelle concentration of the sodium salts of N-acylcysteine, N-acylserine, and N-acylglycine. Recently Ohki and Tokiwa (11) estimated the physicochemical properties of a series of sodium N-acylsarcosinate.

In the investigation of the antimicrobial activities of N-acylamino acids, Kameda et al. (12) reported that N-lauroyl-DL-phenylalanine was strongly sterilizing toward staphylococci. Fosdick et al. (13) found that sodium N-acylsarcosinate had a strong anticaries action. Ueda et al. (14,15), in their patents, described the antivirus action of N-acylglutamic acid. Shimizu and Nauri (16) examined the antimicrobial activities of N-acylamino acids.

Long chain N-acylated compounds of glutamic acid may also have surface activities:



(RCO—long chain acyl radical)

(I)

Kester (17) who first prepared long chain N-acylglutamic acid (I) reported that the salt was useful as a wetting agent, a foaming agent and a detergent. Fieser et al. (18) investigated the emulsifying action of N-stearoyl-L-glutamic acid. Komatsu et al. (19) found that the salts of N-acylglutamic acid had excellent detergency and protected the human skin from the irritation caused by sodium alkylbenzene sulfonate or sodium lauryl sulfate when used as the mixture.

Long chain N-acylamino acids are generally prepared by a reaction of amino acids with fatty acid chlorides or mixed carboxylic-carbonic anhydrides in an aqueous alkaline medium. While these methods are successful in the preparation of N-acyl derivatives of neutral amino acids, they are not suitable for the preparation of long chain N-acyl acidic amino acids such as glutamic acid and aspartic acid. Kester (17) reported in his patent that long chain N-acylglutamic acid could be obtained by the dropwise addition of fatty acid chloride to an aqueous solution of glutamic acid in the presence of potassium hydroxide. But Jungermann et al.

TABLE I

N-Acyl-α-aminoglutarodinitriles

Acyl	Reaction solvent	Crude yield, %	Melting point, C	Analysis, % C Calc	C Found	H Calc	H Found	N Calc	N Found
Lauroyl	Water	96.2	45-47	70.06	69.69	10.03	9.79	14.42	14.47
Myristoyl	Water	63.7	59-62	71.43	71.88	10.41	10.82	13.15	13.59
Palmitoyl	Chloroform	62.7	69-72	72.57	72.71	10.73	10.71	12.09	11.76
Stearoyl	Water	72.0	72-75	73.55	73.53	11.00	11.19	11.19	11.31
Oleoyl	Water	---	Oily						

TABLE II

Variation of Yields of N-Lauroyl-DL-glutamic Acid With Varying Ratios of Acetone and Water

Solvent, ml		Volume %	Yield,
Acetone	Water	of acetone	%
0	60	0	34.6
12	108	10.0	38.0
18	102	15.0	75.7
30	110	21.4	79.3
30	90	25.0	88.8
48	92	34.3	92.1
48	72	40.0	90.0
72	48	60.0	80.2
90	30	75.0	53.5

(20) found that acidic amino acids could not be acylated by this method.

The authors also tried to acylate glutamic acid by Kester's method (17). The resulting yield was only about 35% because of formation of the acid anhydride as a byproduct, obtained by condensation of the carboxylic radical of glutamic acid with fatty acid chloride. Jungermann et al. (20) proposed to reflux a suspension of acidic amino acid and fatty acid chloride in anhydrous ethyl acetate. However, this process yielded only about 30% in the form of N-acylglutamic anhydride. Fieser et al. (18) obtained N-stearoyl aspartic acid by the treatment of N-acylaspargine with sodium nitrate.

It has been a common practice (14,21) to obtain long chain N-acyl acidic amino acid when an acidic amino acid is converted to its dialkyl ester which may thereafter be reacted with an acid chloride in an organic solvent, e.g., chloroform, in the presence of an organic base such as pyridine or triethylamine. Then the resulting N-acyl amino acid dialkyl ester is saponified.

It has also been proposed in some patents that long chain alkylamidine (22), alkyliminoether (22), or carboxylic-carbonic anhydride (18,23) may be used for the acylating agents instead of fatty acid chloride. As another method, Ueda et al. (15) reacted α-pyrrolidone carboxylic acid with lauroyl chloride or lauric acid anhydride to prepare N-lauroyl-α-pyrrolidone carboxylic acid, which thereafter was hydrolyzed with an alkali to obtain N-lauroylglutamic acid. However, the authors were unable to acylate α-pyrrolidone carboxylic acid by this method. In the preparation of long chain N-acylamino acids such as glycine or sarcosine, Shirai et al. (24) described that α-aminoalkylnitrile was reacted with fatty acid chloride in an aqueous alkaline solution to obtain N-acyl-α-aminoalkylnitrile which thereafter was hydrolyzed with an acid or alkaline solution.

The authors have searched for efficient and economical methods for preparing long chain N-acylglutamic acid. In the first method, α-aminoglutarodinitrile (IIIa) which was obtained by the reaction of β-cyanopropionaldehyde (II) with ammonium cyanide was acylated with fatty acid chloride by the Schotten-Baumann method, and N-acyl-α-aminoglutarodinitrile (IV) obtained was hydrolyzed with sodium hydroxide in an aqueous medium under mild conditions to avoid the hydrolysis of an acyl radical. In the other method, long chain N-acyl acidic amino acids were prepared directly from acidic amino acids and higher fatty acid chlorides.

As the result, it was found that the desired N-acyl acidic amino acids could be prepared in high yields by the latter method.

RESULTS AND DISCUSSION

Procedure 1

β-Cyanopropionaldehyde (II) which was prepared by the oxo-reaction of acrylonitrile was reacted with ammonium cyanide in an excess aqueous ammonia solution to obtain α-aminoglutarodinitrile (IIIa), which was an intermediate for the synthesis of glutamic acid and had been isolated as the sulfate (25). The α-aminonitrile could also be isolated as the oxalate (IIIb). The α-aminonitrile was reacted with fatty acid chloride and a base in an aqueous or a chloroform solution to obtain N-acyl-α-aminoglutarodinitrile (IV) in crystalline form. The results are shown in Table I.

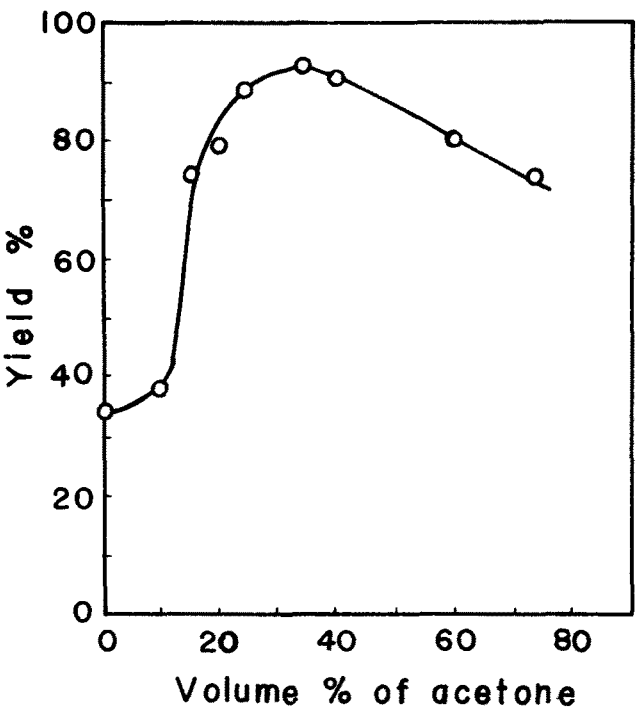
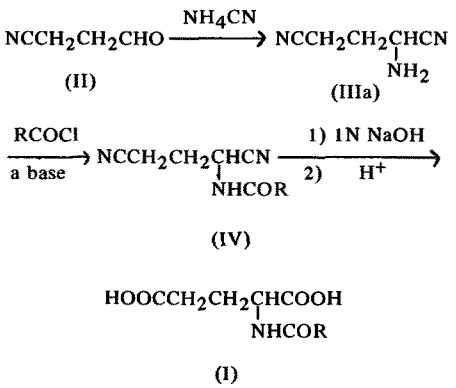


FIG. 1. Relationship between yields of N-lauroyl-DL-glutamic acid and volume per cent of acetone in acetone-water mixture employed as reaction medium.



The N-lauroyl-α-aminoglutarodinitrile was hydrolyzed with 1.1 equivalent amounts of 1N aqueous sodium hydroxide solution to nitrile radicals in an autoclave at 150 C over a period of 30 min to obtain N-lauroyl-DL-glutamic acid in a yield of 80%. When the alkali was added in excess or the hydrolysis was carried out at temperatures above 180 C, the acyl radical of IV suffered from the hydrolysis. When the reaction was carried out at a temperature under 100 C the nitrile radicals were hardly hydrolyzed.

Although this method is very interesting, it has defects in that the α-aminonitrile used as the starting material is unstable and decomposes partially when the excess ammonia containing in the aqueous solution is distilled off, and an optically active N-acylglutamic acid cannot be obtained by this procedure.

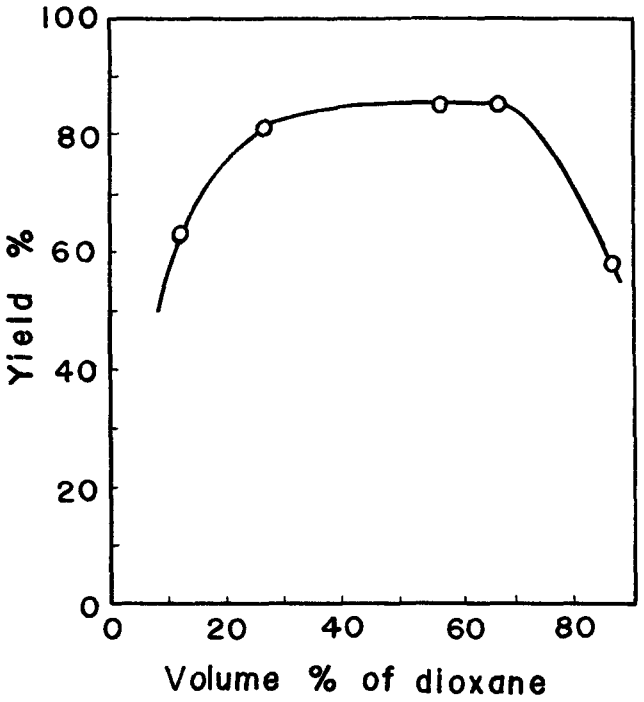


FIG. 2. Relationship between yields of N-lauroyl-DL-glutamic acid and volume per cent of dioxane in dioxane-water mixture employed as reaction medium.

TABLE III

Variation of Yields of N-Lauroyl-DL-glutamic Acid With Varying Ratios of Dioxane and Water

Solvent, ml		Volume % of dioxane	Yield, %
Dioxane	Water		
16	104	13.3	63.2
32	88	26.7	80.8
70	50	58.3	84.3
105	50	67.7	84.3
130	20	86.7	58.2

TABLE IV

Yields of N-Lauroyl-DL-glutamic Acid in Water-Water Miscible Solvent[a]

Organic solvent	Solvent mixture, ml		Yield, %
	Organic solvent	Water	
Tetrahydrofuran	42	58	90.0
t-Butyl alcohol	50	60	77.5
Methyl ethyl ketone[b]	70	70	78.7
Cyclohexanone[b]	70	70	76.0

[a]The experiments were performed under the same reaction condition adopted in the preparation of N-lauroyl-DL-glutamic acid using the dioxane-water mixture as the solvent, as described in Experimental Procedures.

[b]Partially dissolved in water.

Procedure 2

In the condensation of glutamic acid and fatty acid chloride, the reaction was carried out in a mixed solvent of water and a water miscible organic solvent such as acetone, methyl ethyl ketone, dioxane, tetrahydrofuran, t-butyl alcohol or cyclohexanone. Long chain N-acyl acidic amino acids could be prepared in yields greater than 60%, and in some cases maximum yield was about 95%.

$$\mathrm{NaOOCCH_2CH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOONa} +$$

$$\mathrm{RCOCl} \xrightarrow[2)\ H^+]{1)\ NaOH} \mathrm{HOOCCH_2CH_2\underset{\displaystyle NHCOR}{\underset{|}{C}}HCOOH}$$

(I)

Disodium glutamate dissolved in the mixed solvent was reacted dropwise and simultaneously with fatty acid chloride and an aqueous solution of sodium hydroxide under cooling in an ice water bath. After stirring for a while the reaction mixture was acidified with a mineral acid to pH 1 to obtain a crystalline N-acylglutamic acid.

In this condensation reaction the composition of the mixed solvent greatly influences the yield of N-acylated glutamic acid. Table II and Figure 1 show the yield of N-lauroyl-DL-glutamic acid as a function of the composition of a water-acetone solvent mixture employed as a reaction medium. From this result it is evident that the concentration of 30-50% v/v of acetone gives the best yield. Table III and Figure 2 show the same yield as a function of the composition of a water-dioxane mixture. Table IV shows the same yield when water plus other water miscible solvents are used as reaction solvents.

As the acyl radical becomes longer the N-acylation becomes less reactive. Table V and Figure 3 show the yield of N-palmitoyl-DL-glutamic acid when the acylation reaction is performed in an acetone-water mixture of different concentrations at temperatures of 0-5 C. It became evident from this result that the concentration of acetone giving the best yield was very narrow. N-Stearoylglutamic acid was also less reactive and the yield was lower. When the acylation reaction was performed under these reaction conditions the yield was only about 50%. When the acylation reaction was carried out at the higher temperature and at pH 12 the yield was satisfactory. Generally, the better results were obtained when the reaction was carried out under alkaline pH controlled between 11 and 13.

When dimethylformamide was used as the reaction medium the acylation could not be conducted. Acylation was also unsuccessful when water immiscible solvents such as toluene, chloroform, and ethyl acetate were used as the reaction mediums. Long chain N-acylaspartic acid was also obtained in a good yield by the above method.

EXPERIMENTAL PROCEDURES

α-Aminoglutarodinitrile Oxalate (IIIb)

β-Cyanopropionaldehyde (II) (290 g, 3.4 moles) was dissolved in a 4.5 liter of 28% aqueous ammonia solution containing 221 g (4.5 moles) of sodium cyanide and 241 g (4.5 moles) of ammonium chloride. After the reaction mixture was stirred for 1 hr at 22-25 C, the ammonia was removed by distillation in a water bath maintained at 60 C. Ethanol was added to the concentrate to remove the crystalline sodium chloride by filtration and thereafter ethanol was distilled off. Oxalic acid dihydrate (350 g, 2.8 moles) dissolved in hot water was added slowly to this solution and on cooling to 0 C α-aminoglutarodinitrile oxalate (IIIb) crystallized. The crystals were filtered off and dried. The crude yield was 60% (418 g). This oxalate was recrystallized from hot water; mp = 146-147 C. Analysis: Calculated for $C_7H_9O_4N_3$: C, 42.21; H, 4.55; N, 21.10. Found: C, 41.98; H, 4.46; N, 21.40.

N-Lauroyl-α-aminoglutarodinitrile (IVa)

Compound IIIb (71.7 g, 0.36 mole) was suspended in 360 ml of water and 100 ml (0.72 mole) of triethylamine added to form a solution of α-aminoglutarodinitrile (IIIa). While the solution was cooled to 0 C, 78.9 g (0.36 mole) of lauroyl chloride and 14.5 g (0.36 mole) of sodium hydrox-

TABLE V

Variation of Yields of N-Palmitoyl-DL-glutamic Acid with Varying Ratios of Acetone and Water

Solvent, ml			
Acetone	Water	Volume % for acetone	Yield, %
24	88	21.4	57.3
30	70	30.0	64.3
36	64	36.0	94.4
42	58	42.0	92.6
54	46	54.0	73.4
66	34	66.0	44.6

ide in 360 ml water were added dropwise and simultaneously with stirring at pH 9 over a period of about 30 min. The crystals of IVa that precipitated were filtered off, washed with water and dried. The yields were 101 g (96.2%), mp 51-53 C. They were washed in petroleum benzine to remove the free lauric acid and were recrystallized from chloroform-petroleum benzine; mp 45-47 C. Analysis: Calculated for $C_{17}H_{29}ON_3$: C, 70.06; H, 10.03; N, 14.42. Found: C, 69.79; H, 9.79; N, 14.47.

The other acyl aminonitriles (IV) were obtained by the same method and the results are shown in Table I.

Hydrolysis of IVa

Compound IVa (2.9 g, 10 mmoles) was suspended in 22 ml of 1N sodium hydroxide and hydrolyzed for 30 min at 150 C in an autoclave.

The reacted solution was acidified to pH 1 with 42 ml of 1N hydrochloric acid. The precipitated crude crystals of N-lauroyl-DL-glutamic acid were filtered off and dried (3.1 g; mp 81-113 C). They were washed in petroleum benzine to obtain 2.67 g (81.0% yield) of the pure acid and were recrystallized from a mixed solvent of ethanol-water (3:7), mp 115-118 C, neutralization equivalence 97%. Analysis: Calculated for $C_{17}H_{31}O_5N$: C, 61.98; H, 9.49; N, 4.25. Found: C, 62.19; H, 9.71; N, 4.39.

N-Lauroyl-DL-glutamic Acid by Procedure 2

DL-Glutamic acid (14.7 g, 0.1 mole) was suspended in 120 ml of a mixture of 50 ml of water and 70 ml of dioxane, and 8.0 g (0.2 mole) of sodium hydroxide were added to form a solution of disodium DL-glutamate. While the solution was held at 0 C, 6.0 g (0.15 mole) of sodium hydroxide in 20 ml of water and 25.0 g (0.11 mole) of lauroyl chloride were added dropwise and simultaneously with stirring over a period of about 40 min. Stirring at 0 C was then continued for 2 hr. Water (50 ml) and 40 ml of 6N hydrochloric acid were added to the reaction mixture to bring pH 1. The precipitated crude crystals of N-lauroyl-DL-glutamic acid were filtered off, washed with water and dried. The crude crystals (32.4 g) were washed in 300 ml of petroleum benzine and 27.7 g of the pure acid were recovered by filtration (84.3% yield), mp 119.5-121.5 C. The crystals were recrystallized from a mixed solvent of ethanol-water (3:7). Analysis: Calculated for $C_{17}H_{31}O_5N$: C, 61.98; H, 9.49; N, 4.25. Found: C, 62.21; H, 9.70; N, 4.15.

When the ratio of water to dioxane in the reaction medium was varied, the yield varied as shown in Table III and Figure 2.

N-Lauroyl-L-glutamic Acid

A solution of disodium L-glutamate was formed by adding 8.0 g of sodium hydroxide to a suspension of 14.7 g (0.1 mole) of L-glutamic acid in 70 ml of water and 50 ml of acetone.

Sodium hydroxide (6 g) in 20 ml water and 25.0 g (0.11 mole) of lauroyl chloride were added dropwise at 5 C over a period of about 1 hr. Stirring was continued at 0 C for 2 hr and the reaction mixture was acidified to pH 1 with 6N hydrochloric acid to precipitate crystalline N-lauroyl-L-glutamic acid. The crude crystals (32.6 g) were washed with petroleum benzine and 27.6 g of purified N-lauroyl-L-glutamic acid were obtained (yield 84.0%), mp 102-105 C, neutralization equivalence 99.5%, optical rotation $[\alpha]_D^{20} = -10.5$ (C=2, methanol).

When DL-glutamic acid was used instead of the L-glutamic acid in this reaction and the ratio of water to acetone in the reaction medium was varied, the yield varied as shown in Table II and Figure 1.

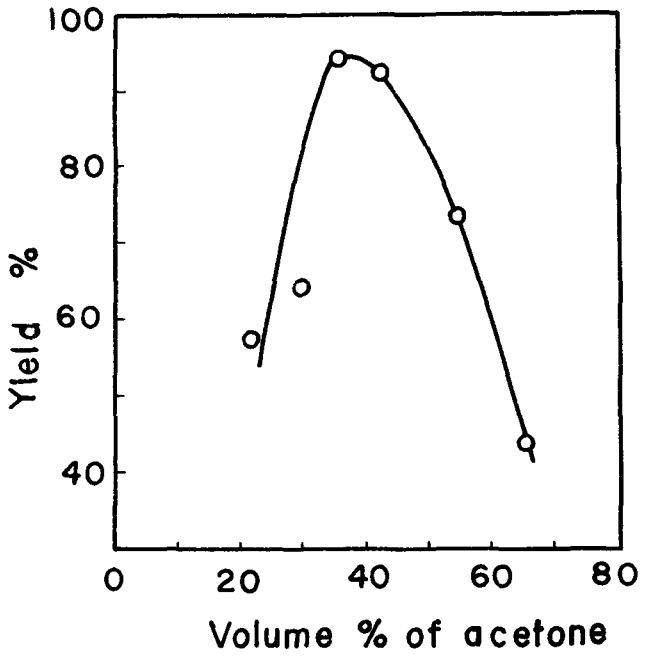


FIG. 3. Relationship between yields of N-palmitoyl-DL-glutamic acid and volume per cent of acetone in acetone-water mixture employed as reaction medium.

N-Palmitoyl-DL-glutamic Acid

A solution prepared from 14.7 g (0.1 mole) of DL-glutamic acid and 8.0 g of sodium hydroxide in 42 ml of acetone and 58 ml of water was mixed with 30.2 g (0.11 mole) of palmitoyl chloride and 6.0 g (0.15 mole) of sodium hydroxide in 20 ml of water at 0-5 C over a period of 30 min. After two additional hours of stirring at 0 C the reaction mixture was diluted with 50 ml of water and acidified to pH 1 to obtain 45.3 g of N-palmitoyl-DL-glutamic acid. The purified N-palmitoyl-DL-glutamic acid (35.7 g) was recovered by washing the crude crystals with petroleum benzine; yield 92.6%, mp 125-128 C, neutralization equivalence 98.3%.

Table V and Figure 3 show the variation in yield with changes in the ratio of acetone to water in the reaction solvent.

N-Stearoyl-L-glutamic Acid

To a solution prepared from 35.3 g (0.24 mole) of L-glutamic acid in 140 ml of water and 120 ml of acetone and 19.2 g of sodium hydroxide, 60.6 g (0.2 mole) of stearoyl chloride and 8.0 g (0.2 mole) of sodium hydroxide in 20 ml of water were added with stirring at 30 C and pH 12 over a period of 20 min. The reaction mixture was stirred one additional hour, cooled, and acidified to pH 1 with sulfuric acid. The precipitated crude crystals of N-stearoyl-L-glutamic acid were washed in petroleum benzine to obtain 74.4 g of the pure crystals (yield 90.0% based on the stearoyl chloride); mp 114-116 C, neutrali-

zation equivalence 100%, optical rotation $[\alpha]_D^{20}$ = -6.3 (C=2, methanol).

N-Lauroyl-L-aspartic Acid

To the solution of 120 ml of acetone and 150 ml of water containing 42.5 g (0.24 mole) of disodium L-aspartate, 8.0 g of sodium hydroxide in 30 ml of water and 43.7 g (0.2 mole) of lauroyl chloride were added over 25 min at 0 C and pH 12.

After further stirring for 30 min the mixture was acidified to pH 1 with sulfuric acid to obtain 58.8 g of crystalline N-lauroyl-L-aspartic acid. When the crude crystals were washed with petroleum benzine, 56.3 g of the purified crystals were obtained (yield 89.0% based on the lauroyl chloride). The crystals were recrystallized from ethanol-petroleum benzine; mp 107-109 C, neutralization equivalence 97.5%, optical rotation $[\alpha]_D^{20}$ = +3.74 (C=2, ethanol). Analysis: Calculated for $C_{16}H_{29}O_5N$: C, 60.93; H, 9.27; N, 4.44. Found: C, 59.96; H, 9.18; N, 4.44.

REFERENCES

1. Bondi, S., Z. Biochem. 17:543 (1909).
2. Abderhalden, E., and C. Funk, Z. Physiol. Chem. 65:61 (1910).
3. Izar, G., Z. Biochem. 40:390 (1912).
4. Karrer, P., E. Miyamichi, H.C. Storm and R. Widmer, Helv. Chim. Acta 8:205 (1925).
5. Hentrich, W., H. Keppler and K. Hintzmann, Ger. Patent 546,942 (1930).
6. Hentrich, W., H. Keppler and K. Hintzmann, Ger. Patent 635,522 (1936); Brit. Patent 459,039 (1936).
7. Staudinger, H., and H.V. Becker, Ber. Deut. Chem. 70:889 (1937).
8. Naudet, M., Bull. Soc. Chim. France 358 (1950).
9. Tsubone, T., J. Japan. Biochem. Soc. 35:67 (1963).
10. Heitmann, P., Eur. J. Biochem. 3:346 (1968).
11. Ohki, K., and F. Tokiwa, J. Japan Oil Chem. Soc. 19:897 (1970).
12. Kameda, Y., E. Toyoura, S. Ohshima, M. Tsuji and C. Irie, J. Pharm. Soc. Japan 68:143 (1948).
13. Fosdick, L.S., J.C. Calandra, R.Q. Blackwell and J.H. Burrill, J. Dental Res. 32:486 (1953).
14. Ueda, T., S. Kato and S. Toyoshima, Japan. Patent 9,568 (1956).
15. Ueda, T., S. Kato and S. Toyoshima, Japan. Patent 1,866 (1958).
16. Shimizu, A., and K. Narui, presented in part at Annual Meeting of Chemical Society of Japan, Osaka, April 1968.
17. Kester, E.B., U.S. Patent 2,463,779 (1949).
18. Fieser, M., L.F. Fieser, E. Toromanoff, Y. Hirata, H. Heymann, M. Tefft and S. Bhattacharya, J. Am. Chem. Soc.78:2825 (1956).
19. Komatsu, S., M. Ishii and T. Nohagi, Japan. Patent 29,444 (1964).
20. Jungermann, E., J.F. Gerecht and I.J. Krems, J. Am. Chem. Soc. 78:172 (1956).
21. Weiss, B., J. Org. Chem. 24:1367 (1959).
22. Ueda, T., S. Kato and S. Toyoshima, Japan. Patent 8,410 (1957).
23. Imoo, S., T. Yamashita, M. Nojima and N. Yano, Japan. Patent 14,004 (1965).
24. Shirai, T., T. Narita, G. Yamaguchi, K. Arai and Y. Kawamura, Japan. Patent 4,710 (1953).
25. Hirahara, T., K. Yarita and T. Akashi, Japan. Patent 8,932 (1966).

[Received September 23, 1971]