

# Use of esters of *N*-hydroxysuccinimide in the synthesis of *N*-acylamino acids

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**ABSTRACT** Several crystalline *N*-hydroxysuccinimide esters of short- and long-chain fatty acids have been synthesized. These compounds react with free amino acids to form preferentially *N*-acylamino acids. The reaction of the *N*-hydroxysuccinimide esters with hydroxylamine and the behavior of the *N*-acylamino acids on thin-layer chromatography are described.

**KEY WORDS** lipoamino acids · *N*-acylamino acids  
· *N*-hydroxysuccinimide esters · thin-layer chromatography

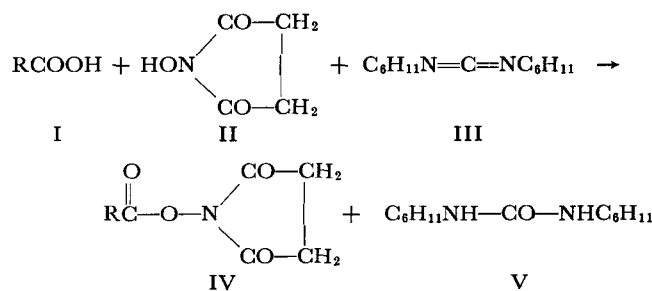
**T**HE FORMATION OF lipoamino acid complexes has been reported in a number of cellular preparations (1). One class of lipoamino acids comprises compounds in which a single amino acid is linked through the amino group to a long-chain fatty acid. Fukui and Axelrod (2) reported a soluble enzyme preparation obtained from rat liver that synthesized *N*-oleoyl and *N*-palmitoyl DL-phenylalanine. It seems probable that an enzymatic synthesis of *N*-acylamino acids is quite widespread in animal tissues and bacteria (1).

Several methods have been used for the preparation of *N*-acylamino acids: lauroyl chloride was allowed to react either with free amino acid (3) or with amino acid esters (4) to form *N*-lauroylamino acids; Ivaldi and Arrigo (5) condensed esters of amino acids with fatty acids in the presence of dicyclohexylcarbodiimide, and fatty acid anhydrides (6) or the mixed anhydride with isobutylcarbonate (7) have also been used for the synthesis of *N*-acylamino acids. As most of the above reactions are carried out in nonaqueous media because of the instability of the acyl chloride or anhydride in water, amino acid esters had to be used in these reactions.

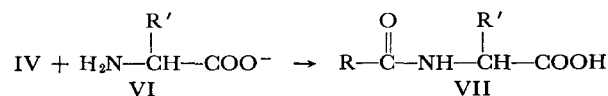
Recently, Anderson, Zimmerman, and Callahan (8) reported the synthesis of *N*-hydroxysuccinimide esters of

acylamino acids and their use in the synthesis of peptides. These esters are crystalline compounds and are highly reactive in nonaqueous as well as in aqueous solutions. Similarly, we prepared the *N*-hydroxysuccinimide ester of acetic acid and used it for the synthesis of *N*-acetyl-phenylalanyl-sRNA<sup>1</sup> (9).

In this communication we describe the synthesis of *N*-hydroxysuccinimide esters of fatty acids (IV) and their use in the preparation of *N*-acylamino acids. The fatty acid (I) is condensed with *N*-hydroxysuccinimide (II) in the presence of dicyclohexylcarbodiimide (III). Dicyclohexylurea (V), which precipitates out, is removed by filtration, and the *N*-hydroxysuccinimide esters of fatty acids (IV) are obtained as stable crystalline compounds in good yield (Table 1).



*N*-Hydroxysuccinimide esters of fatty acids (IV) react readily with the sodium salt of free amino acids (VI) in aqueous solution to form the corresponding *N*-acylamino acids (VII) (Table 2).



Compound IV does not react at all with the carboxyl group of fatty acids to form an anhydride, in contrast to

<sup>1</sup> sRNA, soluble RNA.

TABLE 1 FORMATION OF *N*-HYDROXSUCCINIMIDE ESTERS OF FATTY ACIDS

Acids, Ester of:	Recrystal- lization from:	Yield	mp	Formula	Analysis					
					Calculated			Found		
					C	H	N	C	H	N
		%	°C		%			%		
Acetic	Water	93	130*	C <sub>6</sub> H <sub>7</sub> O <sub>4</sub> N	45.86	4.49	8.91	46.11	4.50	8.92
Caprylic	Ethanol	95	63	C <sub>12</sub> H <sub>19</sub> O <sub>4</sub> N	59.73	7.93	5.80	59.84	7.88	5.85
Lauric	Ethanol	90	75	C <sub>16</sub> H <sub>27</sub> O <sub>4</sub> N	64.61	9.15	4.71	64.60	9.09	4.69
Palmitic	Ethanol	91	90	C <sub>20</sub> H <sub>35</sub> O <sub>4</sub> N	67.95	9.98	3.96	67.76	9.83	4.18
Stearic	Ethanol	94	93	C <sub>22</sub> H <sub>39</sub> O <sub>4</sub> N	69.24	10.30	3.67	69.25	10.17	3.61

\* Melting point 130 °C is reported (10).

TABLE 2 SYNTHESIS OF *N*-LAUROYLAMINO ACIDS\*

	Recrystallization from:	Yield	mp	Formula	Analysis					
					Calculated			Found		
					C	H	N	C	H	N
		%	°C		%			%		
<i>N</i> -Lauroylglycine	Chloroform	83	117†	C <sub>14</sub> H <sub>27</sub> O <sub>3</sub> N	65.33	10.57	5.44	65.24	10.53	5.26
<i>N</i> -Lauroyl-L-serine	Chloroform	78	87	C <sub>15</sub> H <sub>29</sub> O <sub>4</sub> N	62.69	10.17	4.87	62.60	10.11	4.79
<i>N</i> -Lauroyl-L-glutamic acid	Chloroform	81	107‡	C <sub>17</sub> H <sub>31</sub> O <sub>5</sub> N	61.97	9.48	4.25	61.66	9.41	4.35

\* General formula VII.

† Melting point 117–118 °C is reported (5).

‡ Melting point 101–101.5 °C is reported (4).

TABLE 3 HYDROXYLAMINE REACTION OF *N*-HYDROXSUCCINIMIDE AND ITS ESTER

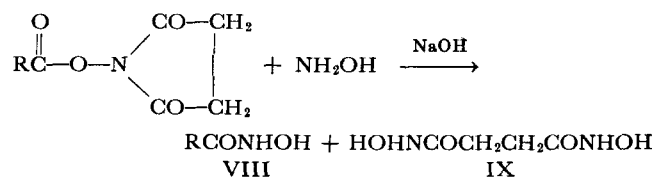
Compound	Amount	Max. Iron- Complex Absorbance at 540 mμ	Hy- droxamic Acid Equiva- lents
Lauric anhydride	0.4	32	1
<i>N</i> -Hydroxysuccinimide	0.4	35	1
<i>N</i> -Hydroxysuccinimide ester of lauric acid	0.4	100	3

Reaction conditions as described in Fig. 1.

acyl chlorides and fatty acid anhydrides, which do react with free fatty acid to form the corresponding symmetrical or mixed anhydrides. When compound IV was mixed with adenosine-5'-phosphate or with cytidine-5'-phosphate, no reaction was detected and the nucleotides remained unchanged. These experiments indicate that *N*-hydroxysuccinimide esters of fatty acids do not react with monoesters of phosphoric acid to form a mixed anhydride, or with hydroxyl groups to form esters, or with the amino group of the adenine and cytosine to form an amide. This finding enables us to perform a specific acylation of an amino acid in the presence of nucleotides, as in aminoacyl-sRNA (9).

Goldenberg and Spoerri (11) reported the reaction between cyclic imides and alkaline hydroxylamine.

According to their results, succinimide reacts with hydroxylamine to form a monohydroxamic acid. We found, by measuring the absorbance of the iron-hydroxylamine complex, that the free *N*-hydroxysuccinimide reacts with hydroxylamine to form the monohydroxamic acid. The *N*-hydroxysuccinimide ester of a fatty acid, on the other hand, reacts with hydroxylamine to form three equivalents of hydroxamic acid (Table 3). It seems probable that in this case the dihydroxamic acid (IX) and the corresponding fatty acid hydroxamic acid (VIII) were obtained.



As can be seen in Fig. 1, Beer's law was obeyed for *N*-hydroxysuccinimide and its ester over the range examined.

## EXPERIMENTAL SECTION

Melting points were determined in capillary tubes by use of the Totalli melting point apparatus. Solvents were dried over molecular sieve (Type 4A 4–8 mesh beads, Union Carbide Corp., Carbon Products Div., N.Y.).

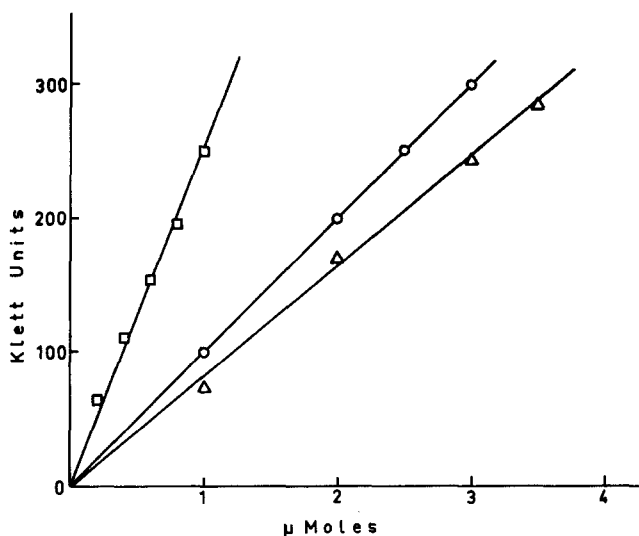


FIG. 1. Hydroxamic acid determination of *N*-hydroxysuccinimide and its ester. 1 ml of a mixture containing equal volumes of 14% hydroxylamine hydrochloride and 14% NaOH in water was added to 4 ml of an ethanolic solution of the sample tested. After 30 min at room temperature, 0.3 ml of 4 *N* HCl was added followed by 1 ml of 5% FeCl<sub>3</sub> in 1.2 *N* HCl (total volume 6.3 ml). The color density was determined in the Klett spectrophotometer using filter No. 54 (Klett Mfg. Co., N. Y.).

○, *N*-hydroxysuccinimide; □, *N*-hydroxysuccinimide ester of lauric acid; Δ, lauric anhydride.

#### *N*-Hydroxysuccinimide Ester of Lauric Acid

Lauric acid (6 g, 30 mmoles) was added to a solution of *N*-hydroxysuccinimide (8) (3.45 g, 30 mmoles) in dry ethyl acetate, (130 ml). A solution of dicyclohexylcarbodiimide (6.18 g, 30 mmoles) in dry ethyl acetate (10 ml) was then added, and the reaction mixture was left overnight at room temperature. Dicyclohexylurea was removed by filtration, and the filtrate was concentrated under reduced pressure to yield white crystals (8.7 g, 95% yield). The crude material contained some dicyclohexylcarbodiimide, acylurea, and traces of lauric anhydride, as could be seen by thin-layer chromatography. Recrystallization from ethanol yielded 8.3 g. (90%) of pure *N*-hydroxysuccinimide ester of lauric acid, mp 75°C. The compound moved as a single spot on a thin-layer chromatogram (Fig. 2).

Other fatty acid derivatives were prepared in a similar manner (Table 1). Each of them moved as a single spot on thin-layer chromatograms with the same relative mobility as *N*-hydroxysuccinimide ester of lauric acid.

#### *N*-Lauroyl L-Serine

A solution of *N*-hydroxysuccinimide ester of lauric acid (300 mg, 1 mmole) in tetrahydrofuran (10 ml) was added to a solution of L-serine (105 mg, 1 mmole) and sodium bicarbonate (84 mg, 1 mmole) in water (10 ml). After 16 hr the solution was acidified to pH 2 with 1 *N* hydrochloric acid and the organic solvent was removed in

vacuo. After addition of water (50 ml) the compound was filtered, dried, and crystallized from chloroform-petroleum ether to give 235 mg (78% yield), mp 87°C.

Other lauroylamino acids were prepared in a similar manner (Table 2). These compounds moved with the same *R<sub>f</sub>* on a thin-layer chromatogram (Fig. 3).

#### Reaction between *N*-Hydroxysuccinimide Ester of Acetic Acid and Adenosine-5'-Phosphate

Adenosine-5'-phosphate (182 mg, 0.5 mmole) was dissolved in dimethylformamide (5 ml) by the addition of tri-*n*-butylamine (0.12 ml, 0.5 mmole). To this solution, the *N*-hydroxysuccinimide ester of acetic acid (241 mg, 1.5 mmoles) was added and the solution was

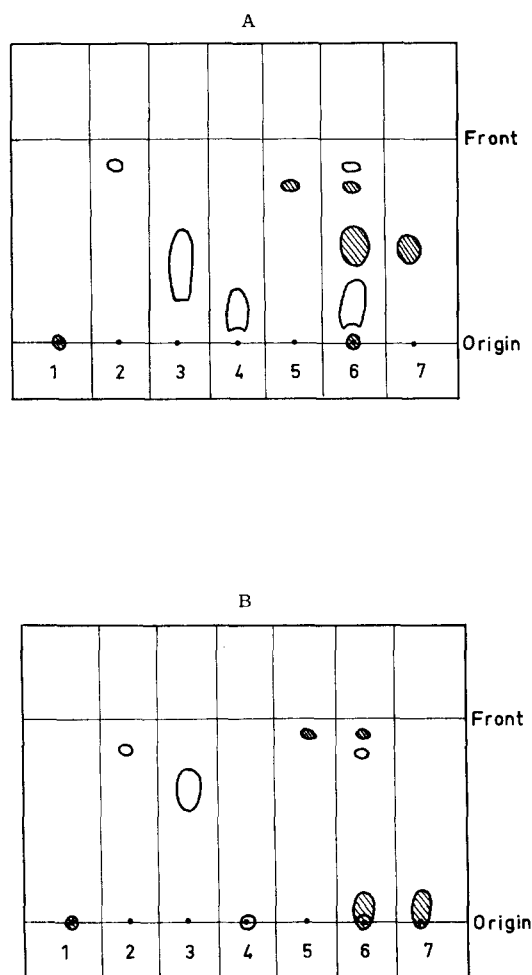


FIG. 2. Thin-layer chromatogram on microchromatoplate coated with silica gel by dipping (13). Solvents: A, chloroform; B, petroleum ether (bp 40–60°C)-diethyl ether 8:2. Indicators: open spots were made visible by charring with 50% aqueous sulfuric acid; striated spots were made visible (red color) by spraying with 14% hydroxylamine solution in water (20 ml)-14% NaOH (8.5 ml), followed after 2 min by spraying with 5% FeCl<sub>3</sub> in 1.2 *N* HCl. 1, *N*-hydroxysuccinimide; 2, dicyclohexylcarbodiimide; 3, lauric acid; 4, dicyclohexylurea; 5, lauric anhydride; 6, crude *N*-hydroxysuccinimide ester of lauric acid; 7, recrystallized *N*-hydroxysuccinimide ester of lauric acid.

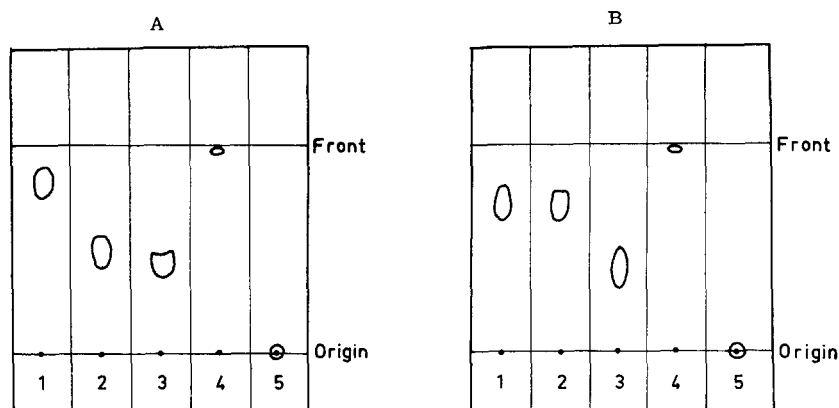


FIG. 3. Thin-layer chromatogram on microchromatoplate coated with silica gel by dipping (13). Solvents: A, methanol-chloroform-acetic acid 0.5:8.5:0.2; B, methanol-chloroform-8 N ammonium hydroxide 3.5:6:0.5. Indicator: charring with 50% aqueous sulfuric acid. 1, lauroylglycine; 2, *N*-lauroyl-L-serine; 3, lauroyl-L-glutamic acid; 4, *N*-hydroxysuccinimide ester of lauric acid; 5, free amino acid (glycine, serine, glutamic acid).

kept at room temperature for 24 hr. The nucleotide was precipitated by adding the reaction mixture to diethyl ether (300 ml), centrifuged, washed several times with acetone, and dried in a vacuum desiccator, to give 165 mg. The ultraviolet absorption (tested by Cary Model 14 Recording Spectrophotometer) was identical with the absorption of adenosine-5'-phosphate;  $\lambda_{\max}$  259  $m\mu$ ,  $\lambda_{\min}$  227  $m\mu$ . On paper chromatography in 1 M sodium acetate-ethanol pH 7.5 (2:5 v/v) it moved as a single spot with the same  $R_f$  as adenosine-5'-phosphate. There was no reaction with hydroxylamine, either at pH 7 or at alkaline pH, which indicates the absence of an anhydride or an ester linkage.

Similar results were obtained when cytosine-5'-phosphate was used instead of adenosine-5'-phosphate.

#### *Reaction between Lauric Acid and N-Hydroxysuccinimide Ester of Lauric Acid*

The *N*-hydroxysuccinimide ester of lauric acid (59 mg, 0.2 mmole) and lauric acid (40 mg, 0.2 mmole) were dissolved in acetone (2 ml). The solution was kept at room temperature for 24 hr, and then analyzed by thin-layer chromatography with ether-petroleum ether

1:4 and chloroform as solvents, according to Selinger and Lapidot (12). No anhydride could be detected, and the only products found were the starting materials.

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