Formation of unusual side-products from succinimidyl esters of fatty acids during the acylation of amino acids[1]

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A new side-product, succinohydroxamic decanoic anhydride, was isolated from the reaction mixture during the acylation of lysine by succinimidyl decanoate in the presence of aqueous sodium hydroxide. Lauric and palmitic acid derivatives were also obtained. It is shown that these compounds can be used for acylation of amino acids. The hydroxamic acid derivatives were also detected when the acylations by succinimidyl esters of fatty acids were carried out under anhydrous conditions. Preparation of *N*-lauroyl-L-tyrosine methyl ester from tyrosine methyl ester and succinimidyl laurate is also described.

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On a isolé un nouveau produit secondaire, l'anhydride succino-hydroxamique décanoique, du mélange réactionnel obtenu lors de l'acylation de la lysine par le décanoate de succinimidyle en présence d'hydroxyde de sodium aqueux. On a également obtenu des dérivés des acides laurique et palmitique. On montre que l'on peut utiliser ces composés pour acyler des acides aminés. On a également décelé des dérivés de l'acide hydroxamique lorsqu'on réalise les acylations des acides gras par les esters succinimidyles en milieu anhydre. On décrit également la préparation de l'ester méthylique de la *N*-lauroyl-L tyrosine à partir du tyrosinate de méthyle et du laurate de succinimidyle.

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There have been several reports in the literature on formation of various side-products arising during the peptide synthesis where the activation of the carboxyl group is promoted by *N*-hydroxysuccinimide. Thus, the presence of succinimidoxycarbonyl-β-alanine-*N*-hydroxysuccinimide ester arising from *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide was observed in some dicyclohexylcarbodiimide/*N*-hydroxysuccinimide mediated couplings (1, 2). Another type of side-product is *O*,*N*-diacylhydroxylamine derivative formed from Boc-proline-*N*-hydroxysuccinimide ester and proline during the coupling of these two amino acids under anhydrous conditions (3).

This paper deals with the side-products arising from succinimidyl esters of fatty acids during the acylation of lysine in the presence of aqueous sodium hydroxide. In the previous communication (4) we have shown that good yields (72–84%) of N^6-substituted lysine derivatives can be obtained by acylation of lysine by succinimidyl esters of fatty acids in water – organic solvent in the presence of triethylamine. The use of aqueous sodium hydroxide as an alternative possibility was described for the work with decanoic acid ester. In such a procedure a large excess of reagent had to be used but the yield was lower (69%). Here we show that the presence of aqueous sodium hydroxide gives rise to mixed succinohydroxamic anhydrides from succinimidyl esters of fatty acids.

The hydrolysis of cyclic *N*-hydroxyimides in the form of their salts to hydroxamic acids has been reported in the literature (5). To our knowledge, this type of hydrolysis has not yet been observed with O-acylated *N*-hydroxyimides. It would be expected that the ester group would undergo saponification under such conditions faster than hydrolysis of the cyclic imide structure. The long chain of fatty acid hinders the ester group and gives rise to a hydroxamic acid derivative instead. In the previously reported syntheses, where excess of triethylamine was used (4), hydroxamic acid derivatives were also formed in all cases. They were separated from the products by extraction with organic solvent and characterized by infrared spectroscopy. Their isolation in pure form could not be achieved. We suggest that precautions should be taken in peptide synthesis when the excess of base is used during work with succinimidyl esters.

Thus, when lysine was acylated by succinimidyl decanoate in 2 *N* sodium hydroxide in dioxane (4), the by-product was isolated in 30% yield by extracting the crude N^6-decanoyl-L-lysine with ethanol. It was crystallized from chloroform–ether and identified as *O*-succinohydroxamic decanoic anhydride (**1**) on the basis of its elemental analysis, infrared and nmr spectra. By treating succinimidyl decanoate with 2 *N* sodium hydroxide in dioxane in the absence of lysine, 56% of the identical com-

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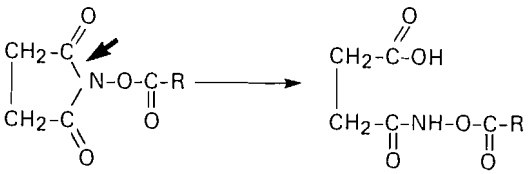

1 R = C_9H_{19}
2 R = $C_{11}H_{23}$
3 R = $C_{15}H_{31}$

pound was obtained, besides decanoic acid, which was easily separated by extraction with chloroform–hexane. This serves as a further proof that the amino acid was not implicated in the formation of this compound.

When lysine was acylated by an excess of succinimidyl palmitate, succinohydroxamic palmitic anhydride (**3**) was isolated in 60% yield, besides the desired N^6-palmitoyl-L-lysine (57%). An identical hydroxamic acid derivative was obtained in 40% yield from succinimidyl palmitate in the absence of lysine. An additional amount of triethylamine was added to the reaction mixture during the work with this reagent. The same conditions served for the conversion of succinimidyl laurate into succinohydroxamic lauric anhydride (**2**) (absence of substrate).

Because hydroxamic acid derivatives have been known as acylating agents useful in peptide synthesis (6–8), it was of interest to explore the possibility of using these side-products for acylation of amino acids. Succinohydroxamic lauric anhydride and β-alanine in water–acetone mixture in the presence of three equivalents of triethylamine gave 76% of *N*-lauroyl-β-alanine. When 1 equivalent of triethylamine or sodium bicarbonate was used, the reaction was slow and impractical. No product was obtained when the acylation was carried out in the presence of 2 *N* sodium hydroxide. This fact explains the reason why these side-products accumulate in the reaction mixture during the acylation of lysine and do not react further with the substrate to give the desired product.

In selective acylations by succinimidyl esters of fatty acids carried out under the anhydrous conditions, namely acylation of the amino group in tyrosine methyl ester in chloroform–triethylamine mixture, only small amounts of hydroxamic acid derivatives could be detected (infrared spectra showed 1800 cm^{-1}, indicating the *O*-acylhydroxylamine bond) even in experiments where excess of reagent was used. The isolation of the side-products in the pure state from these reactions could not be achieved. However, we report in this paper the isolation and structure determination of the main product of the reaction, *N*-lauroyl-L-tyrosine methyl ester, as this structure was unexpected in the light of the results published by other authors. Recently Girin and Shvachkin reported the synthesis of *O*-(Boc-glycyl)-L-tyrosine ethyl ester from L-tyrosine ethyl ester and Boc-glycine-*p*-nitrophenyl ester in chloroform in the presence of an excess of triethylamine (9–11). Upon treating L-tyrosine methyl ester with succinimidyl laurate under such conditions we have obtained *N*-lauroyl-L-tyrosine methyl ester, identified on the basis of its elemental analysis, ir and nmr spectra, and its identity with the compound obtained from the reaction between tyrosine methyl ester and succinimidyl laurate in the absence of base. Its hydrolysis furnished *N*-lauroyltyrosine in 89% yield.

Experimental

Melting points were taken by the capillary method. The nmr spectra were recorded on a Varian T-60 spectrometer. The ir spectra were recorded on a Beckman IR-20 instrument.

Succinimidyl esters of fatty acids were prepared as described previously (4). Amino acids were purchased from Sigma Chemical Company (Saint Louis, Missouri, U.S.A.).

Succinohydroxamic decanoic anhydride (1)

A. *From the acylation of lysine by succinimidyl decanoate*

To a stirred solution of L-lysine hydrochloride (456 mg, 2.5 mmol) in 2 *N* sodium hydroxide (7 mL) was added succinimidyl decanoate (2.01 g, 7.5 mmol) in dioxane (10 mL). A further amount of 2 *N* sodium hydroxide was added to keep the pH at 11–11.5. After 0.5 hour the mixture was acidified with concentrated hydrochloric acid to pH 3 and evaporated to a smaller volume. Then pH was brought up to 6 and the mixture was chilled; the precipitated N^6-substituted lysine derivative was filtered off and washed with water, chloroform–ether (to remove decanoic acid), and with ethanol. The ethanolic extract was evaporated to dryness and the residue crystallized from chloroform–ether to give 735 mg (25%, related to starting succinimidyl ester) of the title compound, mp 153–155°C; ir (Nujol): 1680, 1710–1720, 1795 cm^{-1}; nmr (DMSO) δ: 0.96 (m, 3H, CH_3-decanoic acid), 1.26 (s, 14H, CH_2-decanoic acid), 2.53 (m, 6H, CH_2-succinic acid, α-CH_2-decanoic acid). *Anal.* calcd. for $C_{14}H_{25}NO_5$: C 58.51, H 8.77, N 4.87; found: C 59.32, H 8.49, N 4.80.

B. *From succinimidyl decanoate and aqueous sodium hydroxide*

A solution of succinimidyl decanoate (808 mg, 3 mmol) in 2 *N* sodium hydroxide (3 mL) and dioxane (2 mL) was stirred for 2 hours. The mixture was chilled and acidified to pH 4 and concentrated to a small volume. The solid was separated by filtration, washed with ice-cold water, chloroform–ether mixture (1:4), and recrystallized from chloroform–ether to give 483 mg (56%) of the title compound, mp 155°C. The ir spectrum of this compound was identical with the product obtained from the acylation of lysine.

Succinohydroxamic palmitic anhydride (3)

This compound was obtained from succinimidyl palmitate using procedures A and B described for the preparation of the decanoyl derivative in 60 and 40% yields respectively, mp 153–155°C; ir (Nujol): 1680, 1715–1720, 1795–1800 cm^{-1}; nmr (DMSO) δ: 0.95 (m, 3H, CH_3-palmitic acid), 1.26 (s, 26H, CH_2-palmitic acid), 2.50 (m, 6H, $2CH_2$-succinic and α-CH_2-palmitic acid). *Anal.* calcd. for $C_{20}H_{37}NO_5$: C 64.65, H 10.03, N 3.76; found: C 64.75, H 10.00, N 3.83.

Succinohydroxamic lauric anhydride (2)

This compound was prepared from succinimidyl laurate in 1 mmol scale as described for decanoic derivative (procedure B) to give 162 mg (54%) of the product, mp 152–155°C; ir: 1680, 1710, and 1790 cm^{-1}. *Anal.* calcd. for $C_{16}H_{29}NO_5$: C 60.93, H 9.27, N 4.44; found: C 60.92, H 9.03, N 4.76.

N-*lauroyl-β-alanine*

To a stirred solution of β-alanine (89 mg, 1 mmol) and triethylamine (0.42 mL, 3 mmol) in acetone (1 mL) and water (1 mL) was added 315 mg (1 mmol) succinohydroxamic lauric anhydride. The mixture was stirred overnight, water was added, and concentrated hydrochloric acid to pH 2. The mixture was chilled, the solid separated, washed with water, and recrystallized from ethanol yielding 329 mg (76%) of the title compound. The ir spectrum in Nujol was identical with the spectrum of an authentic sample (1530–1550, 1660–1680 (amide), 1715 (carboxyl)).

N-*lauroyl-L-tyrosine methyl ester in an excess of base*

A solution of 195 mg (1 mmol) of L-tyrosine methyl ester, 297 mg (1 mmol) of succinimidyl laurate, and 1.4 mL (10 mmol) of triethylamine in chloroform (3 mL) was stirred at room temperature overnight (the analysis did not show any laurate present). The mixture was evaporated *in vacuo* till dryness, dissolved in chloroform (5 mL), and washed with 5% hydrochloric acid (2 times, 2 mL), water, and dried over sodium sulfate. The solvent was removed *in vacuo* and the residue was recrystallized from methanol–water and then from chloroform–ether mixture giving 304 mg (80%) of the title compound, mp 82–83°C; $[\alpha]_D^{25}$ +34.5° (*c* 1.0, ethyl acetate); ir ($CHCl_3$): 1510, 1665, 1740 cm^{-1}; nmr ($CHCl_3$) δ: 1.3 (s, 18H), 2.23 (m, 2H), 3.06 (m, 2H), 3.73 (s, 3H), 4.9 (m, 1H), 6.16 (broad s, 1H), 6.9 (m, 4H). *Anal.* calcd. for $C_{22}H_{35}NO_4$: C 69.99, H 9.34, N 3.70; found: C 69.91, H 9.11, N 3.47.

N-*lauroyl-L-tyrosine methyl ester in absence of base*

88% of the product was obtained from this reaction which was carried out as described for the acylation in an excess of base, with the exception that no triethylamine was added and the reaction time was extended to 3 days; mp 83°C; $[\alpha]_D^{25}$ +34.4° (*c* 1.16, ethyl acetate). Infrared and nmr spectra were identical with the product obtained in an excess of base.

N-*lauroyltyrosine*

To a solution of 19.8 mg of *N*-lauroyl-L-tyrosine methyl ester (obtained in the presence of an excess of triethylamine) in methanol (0.2 mL) was added 3% aqueous solution of sodium hydroxide (0.2 mL). The mixture was kept overnight at 0°C, diluted with water, and acidified with concentrated hydrochloric acid to pH 2 and kept at 0°C for two hours. The crystalline solid was collected by filtration, washed with water, and dried, giving 16.8 mg (88%) of the title compound, mp 134–135°C (Koffler block); ir: 1715 (carboxyl), 1660, and 1530 cm^{-1} (amide). *Anal.* calcd. for $C_{21}H_{33}NO_4$: C 69.39, H 9.15; found: C 69.37, H 9.22.

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