

Ammonolysis of Carboxylic Esters Catalyzed by Lipases

Synthesis of Fatty Acid Amides

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INTRODUCTION

Lipases (triacyl glycerol hydrolase, EC 3.1.1.3) are stable and rugged enzymes that act, often enantioselectively, on a wide variety of nonnatural reactants. The exploitation of their characteristic features has become an essential part of the synthetic repertoire.¹ We² and others³ recently reported that ammonia acts as an unnatural acyl acceptor (ammonolysis). Carboxylic esters are converted to the corresponding carboxylic amides in essentially quantitative yield in an exothermic and, hence, irreversible reaction. The lipase from *Candida antarctica*⁴ (B type, Novozym 435) emerged from the initial investigations² as the catalyst of choice.

We wished to extend lipase-catalyzed ammonolysis to the synthesis of fatty acid amides; these are now industrially produced from the fatty acids by a reaction with anhydrous ammonia at approximately 200 °C and 345–690 kPa and are primarily used for their lubricating and surfactant properties.⁵ A low-temperature enzymatic process would seem advantageous for the synthesis of pure fatty acid amides, particularly the thermally unstable unsaturated ones that, *inter alia*, dominate the market for refined fatty acid amides. In the present paper, we report the synthesis of fatty acid amides from lipids as well as from the fatty acids by a one-pot procedure.

MATERIALS AND METHODS

Chemicals and Enzymes

All solvents and reagents were of reagent grade and were dried on molecular sieves before use. Novozym 435 (*Candida antarctica* B lipase on a proprietary carrier, activity of 7550 PLU/g), SP525 (a lyophilized lipase-preparation from *Candida antarctica* B), SP523 (a lyophilized lipase-preparation from *Humicola*), and Novozym IM20 (*Rhizomucor miehei* lipase on anionic resin, activity of 29 BIU/g) were kindly donated by Novo-Nordisk. Lipomax, a lyophilized lipase-preparation from *Pseudomonas alcaligenes*, was kindly donated by Gist-brocades.

SP525, SP523, and lipase from *P. alcaligenes* were immobilized on Accurel EP100 according to a published procedure.⁶ The activities of the resulting preparations

were as follows—SP525: 12.4 kLU/g; SP523: 14.3 kLU/g; Lipomax: 7.5 kLU/g [1 kLU (kilo-Lipase Unit) will liberate 1 mmol of butyric acid from tributyrin at 25 °C and pH 7.2 in 1 min].

Ammonolysis of Lipids

Tributyrin (10 g, 34 mmol), trilaurin (5 g, 7.8 mmol), triolein (5 g, 5.6 mmol), or jojoba wax (5 g, approximately 8.1 mmol) was dissolved in *t*-butyl alcohol (50 mL) that had been saturated with ammonia (2.5 M). Novozym 435 (250 mg) was added and the reaction mixture was shaken at 60 °C until the reaction was judged to be complete by GC (tributyrin) or TLC (other lipids). The reaction products were isolated by standard workup procedures. Full experimental details will be published elsewhere.

Ammonolysis of Butyl Octanoate

Lipase (50 mg) was added to a solution of butyl octanoate (0.5 mL) and diethyleneglycol dibutyl ether (50 μ L, internal standard) in ammonia-saturated *t*-butyl alcohol/*n*-butyl alcohol mixtures (5 mL). The reaction mixture was shaken at 40 °C and the course of the reaction was monitored by HPLC. Results are given later in this report.

One-Pot Ammonolysis of Carboxylic Acids

Lipase SP525 on Accurel EP100 (250 mg) was added to a solution of octanoic acid (5 g, 35 mmol) or oleic acid (5 g, 17.7 mmol) and *n*-butyl alcohol (6 g, 81 mmol) in *t*-amyl alcohol (50 mL). The reaction mixture was stirred for 24 h under reflux at 50 °C *in vacuo*; the refluxing *t*-amyl alcohol was dried over molecular sieves (HPLC yield of butyl octanoate, 98%). The reaction mixture was subsequently saturated with ammonia and stirred at 40 °C until the reaction was judged to be complete by HPLC (168 h). The amides were isolated by standard workup procedures. Full experimental details will be published elsewhere.

RESULTS AND DISCUSSION

Ammonolysis of Lipids

Initial experiments were performed with tributyrin and trilaurin, which were smoothly converted by Novozym 435 into the corresponding amides; in the absence of enzyme, no reaction took place. As an example of long-chain triglycerides, we performed the ammonolysis of olive oil, which consists mainly (approximately 85%) of triolein, because oleamide is one of the most interesting fatty acid amides (see FIGURE 1). The reaction proceeded somewhat sluggishly (72 h at 60 °C for the complete reaction), as might be expected because oleic acid is known to react rather slowly with *C. antarctica* lipase as well as with other lipases.⁷ Nearly pure oleamide

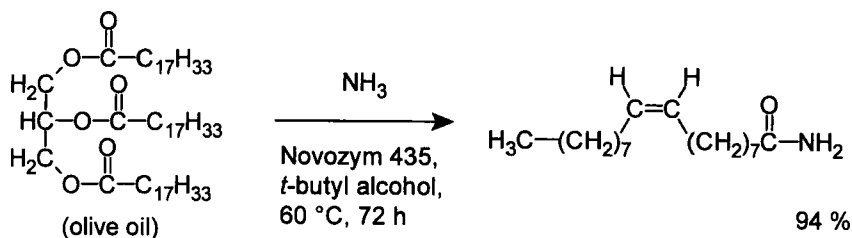


FIGURE 1. Ammonolysis of triolein.

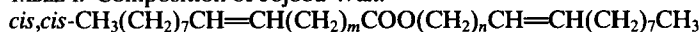
was isolated in about 90% yield; recrystallization afforded pure oleamide in a yield of 82%.

Jojoba wax ester, which is a mixture of long-chain esters (see TABLE 1), is known to resist chemical ammonolysis at the usual conditions of 200 °C and 300–600 kPa.⁸ Ammonolysis of jojoba wax by Novozym 435 resulted in a mixture of fatty acid amides and fatty alcohols after 72 h at 60 °C. Crystallization from hexane afforded the amides (mainly *cis*-11-eicosenamide) in approximately 90% yield. The alcohols, which account for almost 50% of the starting material, were isolated by short-path-length distillation as a 1:1 mixture of *cis*-11-eicosenol and *cis*-13-docosenol.

One-Pot Procedure

Because it is often convenient to start from the carboxylic acid, we have developed a one-pot procedure for the esterification and ammonolysis in the same reaction medium. Octanoic acid was used as a model reactant for the long-chain fatty acids (see FIGURE 2).

The esterification step was straightforward, although removal of water (reflux over 4-Å molecular sieves under reduced pressure) was required for complete conversion. Because a one-component medium would be advantageous in large-scale applications, we first used *n*-butyl alcohol as the reactant and solvent. Ammonolysis was quite sluggish, though, because *n*-butyl alcohol strongly inhibited⁹ Novozym 435 in the ammonolysis step (on the basis of the heats of formation of the reactants and products, ammonolysis would be expected to proceed to completion). The inhibitive effect of *n*-butyl alcohol was further investigated by ammonolysis of butyl octanoate using a number of lipases (see TABLE 2).

TABLE 1. Composition of Jojoba Wax:⁸

		<i>m</i>				<i>n</i>			
<i>m, n</i>		7	9	11	13	8	10	12	14
av. comp. (%)		11	71	14	1	1	45	44	9

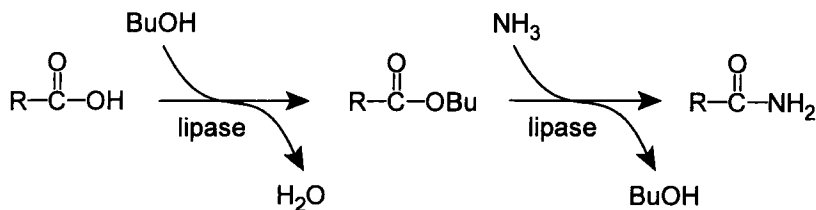


FIGURE 2. One-pot conversion of a carboxylic acid into the amide.

Inhibition of Novozym 435 by *n*-butyl alcohol was avoided by using one equivalent of *n*-butyl alcohol in *t*-amyl alcohol as solvent. Again, water was removed by reflux at diminished pressure over molecular sieves. Butyl octanoate was readily formed (98% in 20 h), but the subsequent ammonolysis was accompanied by 10% hydrolysis to octanoic acid. The problem was traced to the hydrophilic carrier of Novozym 435; this accumulated traces of water in the esterification step, which were released upon saturation of the reaction mixture with ammonia. Accordingly, we next used lipase SP525 (*Candida antarctica* B) immobilized on Accurel EP100 because this very apolar support (polypropylene) would not absorb water. Esterification (using two equivalents of *n*-butyl alcohol) was carried out as before (98% ester in 24 h). Subsequent ammonolysis yielded a 97% yield of octanamide, and only traces of octanoic acid could be detected. After normal workup procedures, octanamide was isolated in 93% yield. By a similar procedure, oleic acid was transformed into oleamide, which was isolated in 90% yield.

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TABLE 2. Ammonolysis of Butyl Octanoate^a

Lipase Source	Conversion (% in 24 h): <i>n</i> -Butyl Alcohol (% v/v in <i>t</i> -Butyl Alcohol)		
	0	50	100
<i>Candida antarctica</i> SP525	99	42	13
<i>C. antarctica</i> Novozym 435 ^b	77	9	5
<i>Humicola</i> SP523	41	27	8
<i>Pseudomonas alcaligenes</i>	80	23	11
<i>Rhizomucor miehei</i> ^b	15	0	0

^aButyl octanoate (0.5 mL), NH₃ (2.5 M), lipase (immobilized on Accurel EP100 unless noted otherwise, 50 mg), solvent (5 mL), 40 °C, and 24 h.

^bImmobilized on a proprietary carrier.

REFERENCES

1. For a review, see: DE ZOETE, M. C., F. VAN RANTWIJK & R. A. SHELDON. 1994. *Catal. Today* **22**: 563–590.
2. DE ZOETE, M. C., A. C. KOCK–VAN DALEN, F. VAN RANTWIJK & R. A. SHELDON. 1993. *J. Chem. Soc. Chem. Commun.*, p. 1831–1832; DE ZOETE, M. C., A. C. KOCK–VAN DALEN, F. VAN RANTWIJK & R. A. SHELDON. 1994. *Biocatalysis* **10**: 307–316; DE ZOETE, M. C., F. VAN RANTWIJK & R. A. SHELDON. 1995. *PCT Int. Appl. WO 95/07359*.
3. GARCIA, M. J., F. REBOLLEDO & V. GOTOR. 1993. *Tetrahedron Lett.* **34**: 6141–6142; CHEN, S-T., M-K. JANG & K-T. WANG. 1993. *Synthesis*, p. 858–860.
4. ISHII, M. (Novo Industri A/S). 1988. *PCT Int. Appl. WO 88/02775*; HELDT-HANSEN, H. P., M. ISHII, S. A. PATKAR, T. T. HANSEN & P. EIGTVED. 1989. *In Biocatalysis in Agricultural Biotechnology*. Chapter 11. ACS Symposium Series Volume 389. J. R. Witaker & P. E. Sonnet, Eds.: 158–172. Amer. Chem. Soc. Washington, District of Columbia.
5. OPSAHL, R. 1992. *In Kirk-Othmer Encyclopedia of Chemical Technology*. Fourth edition. Volume 2. J. I. Koschwitz & M. Howe-Grant, Eds.: 346–356. Wiley. New York.
6. PEDERSEN, S. & P. EIGTVED (Novo-Nordisk A/S). 1990. *PCT Int. Appl. WO 90/15868*.
7. KIRK, O., F. BJÖRKLING, S. E. GODTFREDSEN & T. O. LARSEN. 1992. *Biocatalysis* **6**: 127–134.
8. SHANI, A. 1995. *Chemtech* **52**(5): 49–54.
9. CHULALAKSANANUKUL, W., J. S. CONDORET, P. DELORME & R. M. WILLEMOT. 1990. *FEBS Lett.* **276**: 181–184; RIZZI, M., P. STYLOS, A. RIEK & M. REUSS. 1992. *Enzyme Microb. Technol.* **14**: 709–714; MAXIMO, M. F. & J. P. VAN DER LUGT. 1994. *Biocatalysis* **8**: 321–335; ÖHRNER, N., M. MARTINELLE, A. MATTSON, T. NORIN & K. HULT. 1994. *Biocatalysis* **9**: 105–114; DE GOEDE, A. T. J. W., M. VAN OOSTEROM, M. P. J. VAN DEURZEN, R. A. SHELDON, H. VAN BEKKUM & F. VAN RANTWIJK. 1994. *Biocatalysis* **9**: 145–155; BERGLUND, P., M. HOLMQUIST, K. HULT & H.-E. HÖGBERG. 1995. *Biotechnol. Lett.* **17**: 55–60.