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Erratum

Lipase-catalysed ammoniolysis of lipids. A facile synthesis of fatty acid amides [J. Mol. Catal. B, 1 (1996) 109–113] [1,2]

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Abstract

Ammoniolysis of triglycerides to the corresponding fatty acid amides is efficiently catalysed by *Candida antarctica* lipase (Novozym 435). Thus, olive oil gave 90% of nearly pure oleamide after 72 h at 60°C. Jojoba wax was similarly converted into a mixture of *cis*-11-eicosenamide and erucamide together with *cis*-11-eicosenol and *cis*-13-docosenol.

Keywords: Ammoniolysis; Lipase; Lipids; Fatty acid amides; Amides

1. Introduction

Fatty acid amides are semi-commodities which are produced in thousands of metric tons a year from the fatty acids by reaction with anhydrous ammonia at approximately 200°C and 345–690 kPa [1]; they are primarily used for their lubricating and surfactant properties. The market for refined fatty acid amides is dominated by the unsaturated amides, oleamide (*cis*-9-octadecenamide) and erucamide (*cis*-13-docosenamide), which are used as lubricants in the plastics industry. Owing to their heat sensitivity the manufacture of these amides requires additional distillation steps in order to meet purity specifications. Hence, a low-temperature synthesis which circumvents the need for additional purification is potentially attractive.

Quite recently, oleamide was identified as a natural constituent of the cerebrospinal fluid which induced physiological sleep in rats upon injection [2]. Hence, oleamide has potential applications as a natural sedative.

Another interesting source of unsaturated fatty acid derivatives is jojoba 'oil', which is not a triglyceride but a mixture of long-chain wax esters (see Table 1) [3], consisting mainly of a mixture of the *cis*-11-eicosenyl and *cis*-13-docosenyl esters of *cis*-11-eicosenoic acid and erucic acid. The chemical reactivity of jojoba wax is known to be very low and it resists chemical ammoniolysis under the usual conditions of 200°C at 300–600 kPa. In fact, its transformation into the fatty acid amides is best performed via the methyl esters [4].

The commercial significance of fatty acid amides prompted us to develop a selective, low-temperature synthesis directly from the cor-

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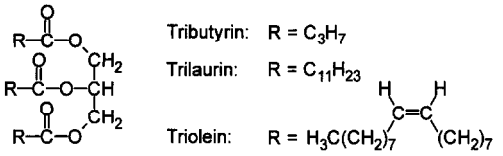

Fig. 1. Structures of the triglycerides.

responding triglycerides (Fig. 1). The lipase-catalysed ammoniolysis of carboxylic esters to their amides, which was reported quite recently, by us [5] and others [6], would seem very promising in this respect. We reasoned that the lipase-catalysed ammoniolysis of triglycerides should take place readily because long chain triglycerides are the natural substrates of lipases. We now wish to report that the lipase-catalysed ammoniolysis of triglycerides and jojoba wax provides a mild and very efficient method for the synthesis of fatty acid amides.

2. Experimental

2.1. Materials and analytical methods

All solvents and reactants were of reagent grade and were dried on activated mol sieves before use. *Candida antarctica* lipase Novozym 435 (EC 3.1.1.3), activity approx. 7550 PLU/g, was kindly donated by Novo-Nordisk A/S (Bagsværd, Denmark). Tributyrin was supplied by Janssen Chimica (Beerse, Belgium). Trilaurin (Fig. 1) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Olive oil was from Aldrich (Bornem, Belgium), jojoba wax from Dekker (Wormerveer, Netherlands).

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ with TMS as internal standard using a Varian VXR-400S spectrometer. Analytical GC using FID was performed on a Packard 428 instrument with a CP-Sil 5CB 10 m × 0.53 mm, df = 5.33 μ column (ammoniolysis of tributyrin) and on a Varian Star 3400CX equipped with a CPSil 5 25 m × 0.32 mm, df = 0.12 μ (ammoniolysis of olive oil and jojoba wax). Mass spectra were recorded using a VG 70-SE mass spectrometer using EI. Melting points are uncorrected and were measured on a Büchi 510 melting apparatus.

2.2. Ammoniolysis of tributyrin

A small-scale test reaction was performed with a solution of tributyrin (500 μl, 1.7 mmol) in ammonia-saturated *t*-butyl alcohol (5 ml, 12.5 mmol NH_3) and diethylene glycol dibutyl ether (250 μl, internal standard). The reaction was initiated by the addition of 100 mg of Novozym 435. The reaction mixture was shaken at 40°C. Aliquots were withdrawn and analysed with GC. After 4 h a complete conversion of the tributyrin into butanamide was observed.

In a preparative-scale experiment tributyrin (10 ml, 34 mmol) was dissolved in ammonia-saturated *t*-butyl alcohol (2.5 M NH_3, 50 ml) and 250 mg of Novozym 435 was added. The reaction mixture was shaken at 60°C during 24 h. The enzyme was then filtered off and the solvent was evaporated in vacuo. The resulting solid was taken up in a small amount of water from which butanamide was isolated by crystallisation and filtration in a yield of 4.74 g, 55%.

2.3. Ammoniolysis of trilaurin

To a solution of trilaurin (5.00 g, 7.82 mmol) in ammonia-saturated *t*-butyl alcohol (50 ml,

Table 1
Composition of jojoba wax [a]

cis,cis-$CH_3(CH_2)_7CH{=}CH(CH_2)_mCOO(CH_2)_nCH{=}CH(CH_2)_7CH_3$

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

[a] Data taken from Ref. [3].

125 mmol NH_3) was added Novozym 435 (250 mg). The reaction mixture was shaken at atmospheric pressure at 60°C during 48 h. The enzyme was then filtered off and the solvent was evaporated in vacuo. The resulting white solid was washed with water and dried at 50°C in vacuo. Yield of laurinamide 4.53 g, 97%; mp: 98–100°C, lit. 101°C [7]. ^{1}H NMR (400 MHz) ($CDCl_3$): δ 0.88 (t, 3H, CH_3), 1.26 (m, 16H, 8 × CH_2), 1.63 (m, 2H, CH_2CH_2CONH$_2$), 2.22 (t, 2H, CH_2CONH$_2$), 5.5–5.8 (d, 2H,CONH_2). ^{13}C-NMR (400 MHz) ($CDCl_3$): δ 14.1, 22.7, 25.6, 29.3, (2 ×), 29.4, 29.5 (2 ×), 29.6, 31.9, 36.0, 175.9. MS: *m/z* (%) 200 (3, M + 1), 170 (4), 156 (3), 142 (2), 128 (6), 114 (8), 100 (3), 86 (11), 72 (51), 59 (100).

2.4. Ammoniolysis of olive oil

To a solution of olive oil (5 g, about 5.6 mmol) in ammonia-saturated *t*-butyl alcohol (50 ml, 125 mmol NH_3) was added Novozym 435 (250 mg). The reaction mixture was shaken at 60°C during 72 h when the reaction was judged to be complete by TLC (silica gel, hexane–ethyl acetate, 10:1). The enzyme was filtered off and the solvent was evaporated in vacuo; the residue was washed with ice–water and dried in vacuo (4.25 g, 94%).

Crystallisation from hexane afforded pure oleamide (3.86 g, 82%), mp 73–74°C, lit. 76°C [8]. ^{1}H-NMR (400 MHz) ($CDCl_3$): δ 0.88 (t, 3H, CH_3), 1.28 (m, 20H, 10 × CH_2), 1.62 (m, 2H, CH_2CH_2CONH$_2$), 2.02 (m, 4H, 2 × CH_2CH=CH), 2.21 (t, 2H, CH_2CONH$_2$), 5.34 (m, 2H, *H*C=C*H*), 5.68, (br. s, 1H, CONH_2), 6.18 (br. s, 1H, CONH_2). ^{13}C-NMR (400 MHz) ($CDCl_3$): δ 14.1, 22.7, 25.6, 27.2 (2 ×), 29.1 29.2, 29.3 (2 ×), 29.5, 29.7, 29.8, 31.9, 36.0, 129.7, 130.0, 176.1. MS: *m/z* (%) 281 (12, M), 264 (6), 238 (5), 220 (3), 198 (3), 184 (4), 72 (83), 69 (27), 59 (100), 55 (53).

2.5. Ammoniolysis of jojoba wax

Ammoniolysis of jojoba wax (5 g, about 8.1 mmol) was carried out as above. The crude product (5.25 g) afforded upon crystallisation from hexane a mixture of the amides (2.45 g, approx. 90%) which contained mainly *cis*-11-eicosenamide (62.6%), together with minor amounts of erucamide (10.4%) and oleamide (9.3%) according to GC. Further crystallisation from acetone afforded a somewhat purer sample which consisted of *cis*-11-eicosenamide (77.6%), erucamide (13.1%) and oleamide (6.9%).

The hexane solution (vide supra) yielded 2.45 g of an oil upon evaporation of the solvent. A part of this residue (0.82 g) was subjected to bulb-to-bulb distillation in a Büchi Kugelrohr apparatus. The distillate (0.59 g, 72%) consisted of *cis*-11-eicosenol (45.6%), *cis*-13-docosenol (45.6%) and *cis*-15-tetracosenol (7.9%).

2.5.1. Identification of cis-11-eicosenamide

^{1}H-NMR (400 MHz) ($CDCl_3$): δ 0.88 (t, 3H, CH_3), 1.27 (br. s, 24H, 12 × CH_2), 1.62 (m, 2H, CH_2CH_2CONH$_2$), 2.01 (m, 4H, 2 × CH_2CH=CH), 2.21 (t, 2H, CH_2CONH$_2$), 5.34 (m, 2H, *H*C=C*H*), 5.62, (br. s, 1H, CONH_2), 6.08 (br. s, 1H, CONH_2). ^{13}C-NMR (400 MHz) ($CDCl_3$): δ 14.1, 22.7, 25.6, 27.2, 29.3, 29.4, 29.5 (2 ×), 29.8, 31.9, 36.0, 129.9 (2 ×), 176.1. GC–MS: *m/z* (%) 309 (12, M), 292 (7), 266 (3), 248 (1), 226 (1), 212 (2), 154 (3), 140 (3), 72 (84), 69 (26), 59 (100), 55 (48).

2.5.2. Identification of erucamide

GC–MS: *m/z* (%) 337 (7, M), 320 (5), 72 (64), 69 (32), 59 (100), 55 (54).

2.5.3. Identification of the alcohols

^{1}H-NMR (400 MHz) ($CDCl_3$): δ 0.88 (t, 3H, CH_3), 1.28 (br. s, 28H, CH_2), 1.58 (m, 2H, CH_2CH_2OH), 2.02 (m, 4H, 2 × CH_2CH=CH), 3.63 (t, 2H, CH_2OH), 5.34 (m, 2H, *H*C=C*H*). The signal at δ 1.28 corresponds to 14 × CH_2, which agrees with a 1:1 mixture of C_{20} and C_{22} alcohols. ^{13}C-NMR (400 MHz) ($CDCl_3$): δ 14.1, 22.7, 25.8, 27.2, 29.3, 29.5 (2 ×), 29.6 (2 ×), 29.7 (3 ×), 29.8, 31.9, 32.8, 63.1, 129.8, 129.9 (4 ×). GC–MS (*cis*-11-eicosenol): *m/z* (%)

278 (18, M − 18), 250 (4), 236 (2), 222 (3), 208 (3), 194 (4), 180 (5), 166 (7), 152 (10), 138 (18), 124 (22), 110 (44), 96 (98), 82 (100), 69 (98), 55 (100). GC–MS (*cis*-13-docosenol): *m/z* (%) 306 (14, M − 18), 278 (3), 264 (2), 250 (3), 236 (2), 222 (2), 208 (3), 194 (4), 180 (5), 166 (6), 152 (8), 138 (15), 124 (23), 110 (34), 96 (82), 82 (56), 69 (82), 55 (100). GC–MS (*cis*-15-tetracosenol): *m/z* (%) 334 (8, M − 18) 306 (3), 278 (3), 264 (2), 250 (3), 236 (2), 222 (3), 208 (2), 194 (3), 180 (4), 166 (4), 152 (7), 138 (9), 124 (13), 110 (33), 96 (64), 82 (72), 69 (67), 55 (100).

3. Results and discussion

The catalyst that we used in the ammoniolysis experiments was the recombinant *Candida antarctica* B lipase (Novozym 435) [9], which has emerged from our previous work on enzyme-catalysed ammoniolysis [5] as the catalyst of choice for this kind of reaction. Initial experiments were performed with tributyrin because its conversion could easily be monitored by GC. At 40°C and atmospheric pressure in ammonia-saturated *t*-butyl alcohol (2.5 M NH_3), Novozym 435 smoothly converted tributyrin via di- and monobutyrin (see Fig. 2). After 4 h reaction time tributyrin was completely converted into butanamide and glycerol; in the absence of enzyme no reaction took place. A preparative scale reaction allowed for the isolation of butanamide. The isolated yield was only moderate (55%), however, because glycerol was difficult to remove from the butanamide, but this problem was not expected to play a role with long-chain fatty acid amides (Fig. 3).

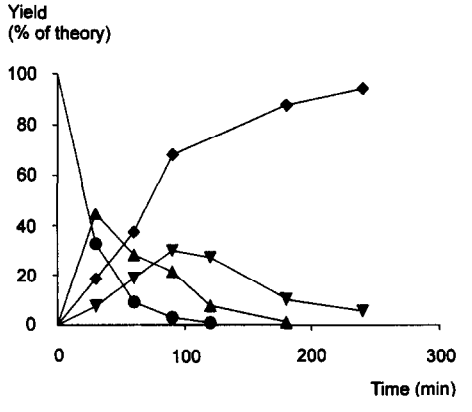

Fig. 2. Ammoniolysis of tributyrin catalysed by Novozym 435. ● Tributyrin, ▲ dibutyrin, ▼ monobutyrin, ◆ butanamide. Further details are given in Experimental.

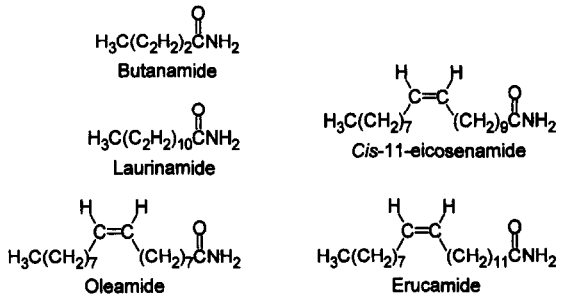

Fig. 3. Structures of the fatty acid amides.

Trilaurin, which is commercially available as a pure compound, was used as a model compound for long-chain triglycerides. Because its reaction could not be monitored with the GC equipment at our disposal only a preparative scale reaction was performed which afforded laurinamide in 97% yield. Next we turned to the long-chain triglycerides and, because oleamide is one of the most interesting fatty acid amides, performed the ammoniolysis of olive oil, which consists mainly of triolein (Fig. 1). The reaction proceeded somewhat sluggishly (72 h, 60°C) as might be expected, because oleic acid is known to react rather slowly with *C. antarctica* lipase as well as with other lipases [10]. Nearly pure oleamide was isolated in about 90% yield; recrystallisation afforded pure oleamide in a yield of 82%.

Ammoniolysis of jojoba wax by Novozym 435 afforded a mixture of fatty acid amides and fatty alcohols after 72 h at 60°C. Crystallisation from hexane afforded the amides (mainly *cis*-11-eicosenamide) in approx. 90% yield. Isolation of the alcohols, which account for almost 50% of the starting material, was somewhat more laborious. Short-pathlength distillation of

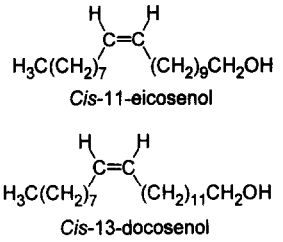

Fig. 4. Structures of the fatty alcohols from jojoba wax.

a sample gave a 1:1 mixture of *cis*-11-eicosenol and *cis*-13-docosenol (structures Fig. 4).

4. Conclusion

Ammoniolysis of long chain triglycerides and of jojoba oil provides a potentially attractive alternative method for the synthesis of fatty acid amides under mild reaction conditions in high yields.

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