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Neolipid Enzymatic Synthesis

Acylated Aminopolyols

JOSE D. FONTANA,*[,1] ROLAND BECK,[2] MADALENA BARON,[1] EDUARDO R. A. DE ALMEIDA,[1] AND EVERSON NOGOCEKE[3]

[1]*LQBB, Department of Biochemistry, Federal University of Parana (UFPR), P.O. Box 19046, (81.531) Curitiba, Parana, Brazil;* [2]*Cerestar R&D, Vilvoorde, Belgium; and* [3]*CPGTQ, Department of Chemical Technology, UFPR*

ABSTRACT

Enzymatic coupling of aminopolyols and medium-chain length fatty acids was carried out via the reverse action of a fungal lipase. Model reactants and catalysts were glucamine (1-amino-1-deoxysorbitol = AmS), pelargonic (nonanoic) acid, and Lipozyme IM-20. These reactants were selected since aminosorbitol is both a precursor for deoxynojirimycin (dietetic inhibitor for glucosidase) and for *N*-methylglucamine (an antileishmanial drug when complexed with antimony), and pelargonic acid is known to generate biotin-vitamers in some bacteria. The reaction proceeded in the presence of dry or water-saturated apolar organic solvents such as hexane and carbon tetrachloride. Increased molar ratio of acyl donor to acyl acceptor allowed the esterification and the amidation reactions to proceed with no need of solvent addition. A broad specificity was found for Lypozyme reverse action in terms of both acyl acceptors and donors.

Index Entries: Aminopolyols acylation; *Mucor* lipase; neolipid synthesis; nonanoyl-sorbitilamide; glucamine nonanoyl-ester.

Abbreviations: AmS = 1¯amino-1-deoxy-D-glucitol or glucamine; mAmS, eAmS, and heAmS = 1-*N*-methyl-, 1-*N*-ethyl, and 1-*N*-hydroxyethyl-aminosorbitol; AmS.HCl = aminosorbitol hydrochloride; AmG = 1-amino-1-deoxy-D-galactitol; C-7 and C-8 = heptanoic and octanoic acids; C-9 = nonanoic or pelargonic acid; dSa = disorbitilamine;

*Author to whom all correspondence and reprint requests should be addressed.

LPZ = Lipozyme IM-20, a fungal lipase; TLC and HPTLC = thin layer chromatography and high performance TLC; GLC and GLC/MS = gas liquid chromatography and GLC coupled to mass spectrometry; FID = flame ionization detector; BSTFA = bistrifluoroacetamide; NMR = nuclear magnetic resonance; TFA = trifluoroacetic acid; R_f = resonance to the solvent front; R_T = retention time; e.i. = elektron impact.

INTRODUCTION

The esterase activity of the fungus *Mucor miehei,* that is, its natural catalytic role using water as cosubstrate, has been used to enhance the flavor of plant and animal fats (*1*). More recent uses of the same lipase were the amidation of long-chain (C-18) fatty acid with middle-chain free amine (C-12) (*2*) and butylamine aminolysis of fatty acid methyl esters (*3*). In comparison, *Aspergillus niger* lipase is able to transfer the acyl moiety of the activated donor butyril trichloroacetate to both active positions of the bifunctional acceptor 6-amino-1-hexanol, but ester formation proceeds 37 times faster than that for the amide (*4*). Two remarkable features of lipase reverse action are the porcine pancreatic enzyme stability in organic solvents even at 100°C (*5*), and the 100% conversion of oleic acid and ethanol to ethyl oleate by *M. miehei* enzyme (*6*). A revolutionary advance dealing with applied lipidology was the region-selective esterification of carbohydrate and carbohydrate-containing structures by the reverse action of a bacterial protease, namely subtilisin (*7*).

Since aminosorbitol and pelargonic acid already display interesting biological activities, the former as precursor of deoxynojirimycin (dietetic inhibitor of α-glucosidase) and of meglumine or *N*-methyl-D-glucamine (antileishmanial drug once complexed with antimony), and the C-9 acid as a biotin-vitamer precursor in bacteria (*8*), we decided to investigate the ability of immobilized *Mucor miehei* lipase (Lipozyme IM-20, specific for the 1,3 transesterification of propanetriol) in carrying out the acylation of the bifunctional carbohydrate aminosorbitol with free medium size-chain length fatty acids such as the C-7 to C-9 members of the saturated series, looking for its respective ester and amide derivatives. Special attention was paid to the possibility of omitting any added organic solvent to the lipase reverse action through the increase of the molar proportion of the acyl donor reactant, taking advantage of nonanoic acid to be the largest fatty acid still liquid at temperatures <30°C (m.p. 12.4°C). Lipase specificity towards both acyl acceptors and donors was also examined.

MATERIALS AND METHODS

Reagents

Aminosorbitol or glucamine (1-amino-1-deoxy-D-glucitol; >98% degree purity) and related acyl acceptors were a kind gift from Cerestar R&D

(Vilvoorde, Belgium). Lipozyme IM-20 was provided by Novo Nordisk (Curitiba-PR, Brazil). Moisture (and eventual colored matter) from pelargonic acid (Merck), heptanoic, and octanoic acids (Aldrich) was removed through molecular distillation in a Kontes apparatus.

Standard Incubation

Aminopolyol, fatty acid donor, and lipase (0.5 mMol, 0.5 to 3.0 mMoles and 90 mg of macroporous resin-immobilized lipase, respectively) were thoroughly mixed with the help of a magnetic bar, and anhydrous or water-saturated organic solvent added till a 2.0 mL final volume in a capped reacti-vial was reached. For 48 h incubation, temperature in the 35°C range was maintained by a hot plate stirrer. The reaction was stopped by filtering the mixture through a tight glass wool pad and combining the filtrate with the subsequent residue washings with 1 vol of each methanol and dioxane. Prior to GLC derivatization or spectroscopic analyses, product samples were dried in a model SC-100 vacuum centrifuge (Savant, Farmingdale, NY) with medium heating.

Analytical Procedures

Thin-layer chromatography (TLC) analyses were carried out on silica gel 60 with aluminum foil chromatoplates (Merck's art. 5553) or with HPTLC F_{254} glass chromatoplates (Merck's art. 5628) using isopropanol : nitromethane : ethyl acetate : ethylmethylketone : methanol : water (50 : 45 : 50: 25: 10 : 20) as mobile phase and a sequential revelator schedule with fluorescamine (for free R-NH_2 groups), rhodamine (for general lipids), alkaline permanganate (for polyol structure), and/or potassium dichromate + sulfuric acid (for less volatile compounds) (spray formulations as (*9*)). Melting points were obtained in an automatic model FP-80 apparatus (Mettler, Sao Paulo, Brazil). A HP-5 column (25 m. crosslinked 5% phenylmethylsilicone, from Hewlett-Packard) with N_2 as carrier gas and a FID detector was used for the GLC capillary analyses of persilyl derivatives (pyridine : BSTFA, 1:1, 70°C, 30 min) of reactants and products, and of phenyl-β-D-galactopyranoside as internal standard. GLC-MS of the ester product, in the direct inlet mode, was carried out with an underivatized sample in a model QP 2000-A apparatus (Shimadzu, Kyoto, Japan). HPLC runs were performed in standard Novapak C-18 and Fatty Acid columns (Waters) isocratically and respectively eluted with acetonitrile : tetrahydrofuran : methanol (45 : 20 : 30) diluted 2 : 3 with distilled water (flowrate = 0.75 mL/min; 642 psi) and water (containing 0.5 mL% of sat. ammonium acetate) : acetonitrile : dioxane : methanol (50 : 20 : 20 : 10), final pH adjusted from 6.7 to 4.0 with acetic acid (flow rate = 0.8 mL min; 820 psi). The bimodular SC 600E/WISP 712 machine was equipped with an 410 DR refraction index detector set at sensitivity 20 and scale factor 40. ^{13}C-NMR spectra of the reactants and of the amide product were

obtained in an AC-300-P machine (Bruker, Germany) using a 5-mm diameter tube and 60 mg sample with d_6-DMSO as solvent and internal standard for the chemical shifts recording (δ, ppm). Acid hydrolyses of the crystalline amide product were carried out with 0.1 or 4.0*M* trifluoroacetic (TFA) acid at 95°C for 15 min.

RESULTS AND DISCUSSION

In order to ensure a convenient and fast method of lipase reverse action monitoring, a solvent mix based in several organic solvents was designed and used as mobile phase for TLC chromatoplates. The isolated or sequential use of selective spray reagents facilitated the recognition of reactants and products. As shown in Fig. 1 (A and B), differential staining with fluorescamine followed by plate inspection under UV light (short and/or long wavelength) selectively revealed (Fig. 1A): aminosorbitol, at Rf = 0.015, a main ester product (that is a free -NH_2 "greasy" product), at Rf = 0.21, and trace amounts of a second product with a Rf = 0.55 (probably a diester, since it stains with both revelators). The second spray with rhodamine confirmed the previous assigned spots and further revealed a second major product with Rf = 0.69 (that is, a neolipid product blocked at the -NH_2, hence an amide); also seen, pelargonic acid (as well as heptanoic and octanoic acids) as reagents excess (at Rf = 0.92) and the run marker (dodecyl-β-D-glucopyranoside at Rf = 0.83). Chromatographic data showed a similar product generation profile for AmS + heptanoic acid, regardless of the solvent condition used (anhydrous or water-saturated carbon tetrachloride; Figs. 1A and 1B, lanes II and III). Also worth mentioning was the similar product yield provided by Lipozyme action even in the absence of any added solvent (same Figs. lane IV). Excess of water, resulting in a two phase system, leads to neither net synthesis of the ester nor of the amide products (lane I). In all positive reactions, including those where octanoic acid (lane V) or nonanoic acid (lane VI) substituted for heptanoic acid (lanes II to IV), the slower moving product ((mono)ester at Rf = 0.20) was dominant over the faster moving one (amide at Rf = 0.69). Aminosorbitol esters from C-7, C-8, and C-9 acyl donors could not be well separated in TLC plates, but were resolved in the GLC runs (as commented on later). Using the same TLC conditions, other kinetic parameters were examined. As shown in Figs. 2A and 2B, the whole material from the incubation (lane T) could be reasonably purified by using silicic acid column chromatography: Chloroform washed out the excess of unreacted pelargonic acid (lane C), acetone eluted most of ester (low Rf) and amide (high Rf) products (lane A), and the following elution steps with methanol (lane M) and water (lane W) completed the recovery of the amide. In the same plates, the double revelator application (fluorescamine followed by rhodamine) allowed to follow the time

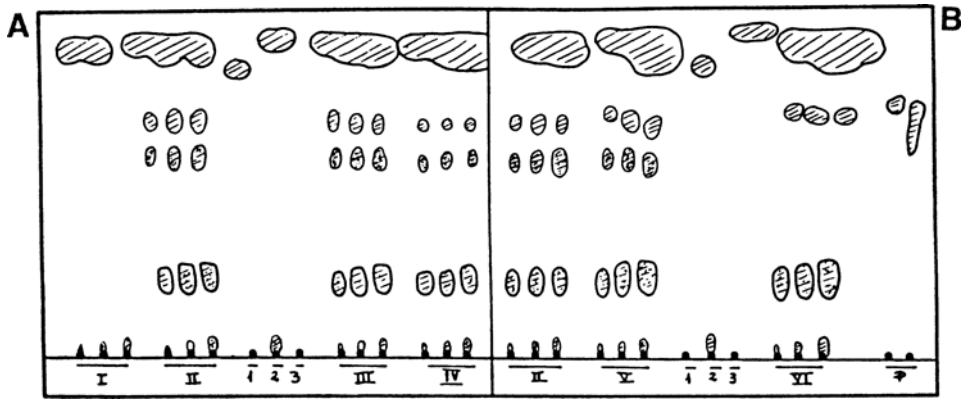

Fig. 1. TLC analysis for water content and fatty acid chain length effects.

Symbols:

AmS	= aminosorbitol	
C-7, C-8, and C-9	= heptanoic, octanoic, and nonanoic acids	
LPZ	= Lipozyme IM-20	
I	= LPZ + AmS + C-7	: carbon tetrachloride + water (2 : 1)
II	= idem	: anhydrous carbon tetrachloride
III	= idem	: water-saturated carbon tetrachloride
IV	= idem	: no solvent addition
V	= LPZ + AmS + C-8	: anhydrous carbon tetrachloride
VI	= LPZ + AmS + C-9	: idem
	(molar ratio for AmS: fatty acid = 1 : 6 in reactions I to VI)	
P	= pearled product crystallized from reaction VI	

Standard

1 = dodecyl-β-D-glucopyranoside
2 = aminosorbitol
3 = pelargonic (C-9) acid

Revelators:

= fluorescamine (+) reaction

= rhodamine (+) reaction

= charring (+) reaction

(The superior line of each box corresponds to the solvent migration front).

course generation of products from AmS + C-9 (lanes 7, 20, and 29 as hours of incubation at 35°C in anhydrous CCl_4): in the shorter time only the ester was detectable and its amount progressively increased from 7 to 29 h; in the longer incubation time, some of the amide product was also visible. This product generation profile is quite different if one compares the 48 h incubation for reactions where reactants ratio aminosorbitol : pelargonic acid (Ams : C-9) was kept as 1: 1 or 1 : 6 (lanes with these same

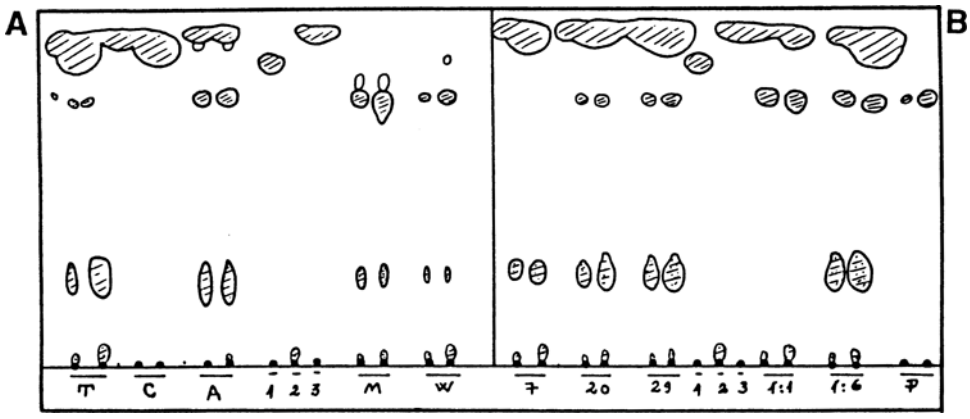

Fig. 2. TLC analysis for time course of acylation, reactants ratio effect and neolipids fractionation profile.

T = enzyme-free mixture of the reactants and the products from reaction VI.

C, A, M, W = chloroform, acetone, methanol, and water eluates from silicic acid column after application of sample T from reaction VI

7, 20, 29 = samples from reaction VI after the indicated hours of incubation at 35°C

1 : 1 = LPZ + [AmS + C-9 = 1 : 1]; 48 h at 35°C in anhydrous CCl_4

1 : 6 = idem [1: 6]; idem

(Other details as in Fig. 1.)

labels). The equivalent ratio led to exclusive amide synthesis, whereas the higher proportion of acyl donor generated both the amide and ester, as well trace amounts of a third component with an average Rf = 0.57 (probably the diesterified form of the aminosorbitol, instead of monoesterified form of the amide, since it was also detectable by the fluorescamine spray). Solvent nature effect on the synthesis of the ester product is depicted in Fig. 3. The fast rate of synthesis was attained using hexane (two upper curves, for AmS : C-8 ratios = 1 : 6 and 1 : 12) as compared to nitrobenzene (two lower curves). Taking into consideration the respective hydrophobicity of these two solvents (log P = 3.5 and 1.8) (*10*), a parallel experiment with acetonitrile (AmS : C-8 ratio = 1 : 6) resulted in a curve positioned in the middle position of the four curves depicted in Fig. 3. Acetonitrile is by far more polar than the above solvents (log P = −0.33). The routine assays with CCl_4, a less expensive solvent, (lop P = 3.0), gave similar results to those obtained with hexane. Applying the routine incubation conditions but enlarging the reactional volume by adding 2–4 times more solvent and/or extending the incubation time (> 72 h), or raising the incubation temperature from 35°C to 45–55°C, the estimated best yield for both products (ester + amide) was in the range of 40–50% of all aminosorbitol initially available. Up to 45°C, no product was detectable in

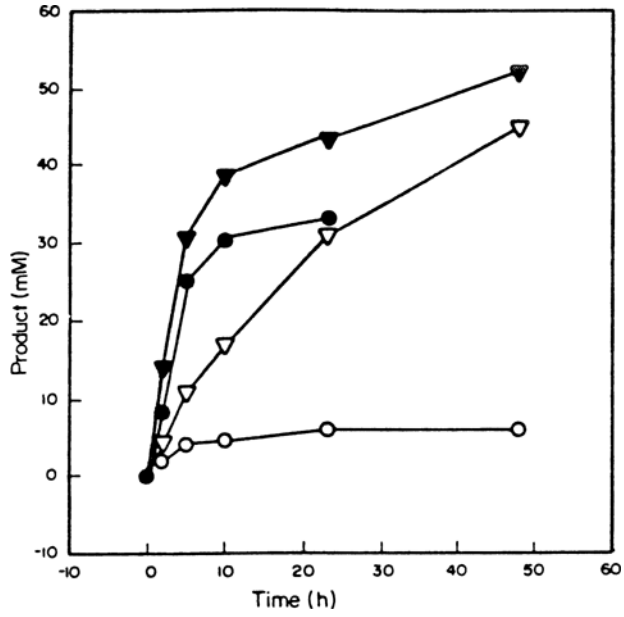

Fig. 3. Solvent effect on the octanoylation of aminosorbitol. Duplicate reactions of 36 mg of Aminosorbitol + 380 or 190 μL of octanoic acid + 40 mg of Lipozyme + anhydrous solvent to the final vol of 2.0 mL were incubated at 25°C till 48 h. Samples, previously persilylated, were analyzed by GLC on the HP-5 column.

——▼——▼—— = hexane (C-8 donor = 1.2*M*)
——●——●—— = idem (idem = 0.6*M*)
——△——△—— = nitrobenzene (idem = 1.2*M*)
——○——○—— = idem (idem = 0.6*M*)

any of the blank assays, that is, omitting, in each case, the enzyme or one of the reactants. However, in 48 h at 50°C (results not shown) some amount of ester product was formed because of the anhydrous condition used and the temperature increase. Interestingly, progressive dilution of reactants with CCl_4 and/or temperature increase to 40°C resulted in complete solubilization of the reagents mix, that is, the biphasic nature of the reaction was dictated just by the enzyme (a particulated catalyst) addition.

Once it was found that Lipozyme was able to transfer C-7 and C-8 acyl residues to aminosorbitol (AmS), a second possibility of its broad specificity was approached. This concerned the nature of aminopolyol acceptor structure. Consequently, in order to evaluate the effect of a secondary amine acceptor group or the effect of an surplus -OH group, the 1-*N*-methyl-(mAmS; meglumine, an anti-leishmanial drug when complexed with antimony), 1-*N*-ethyl-(eAmS), and 1-*N*-hydroxyethyl-aminosorbitol (heAmS), were assayed under the standard incubation conditions (acceptor : C-9 donor = 1 : 6, anhydrous CCl_4; 24 h, 37°C). In addition, 1-amino-

1-deoxy-D-galactitol (AmG) and disorbitilamine (dSa, a dimeric form of aminosorbitol offering a secondary amine acceptor with two polyalcoholic tails) were assayed. Results seen by TLC (sequential fluorescamine→rhodamine→acid dichromate charring; below indications between bars as (+) and (−) reactions) revealed an interesting agreement with the theoretical expectation. Taking the aforementioned profile seen for the AmS+C-9 reference incubation, that is, in order of increasing Rf in TLC: (mono)ester (=0.25; /+/+/+/), probable (di)ester (=0.75; /+/+/+/), and amide (=0.86; /−/+/+/), the additional results were as follows:

1. Methylation of AmS at 1-N resulted in a single product with ester reactivity [(low Rf; (−) reaction for fluorescamine], a similar profile observed for eAmS with a slightly high Rf;
2. Hydroxylation at the ethyl chain of eAmS resulted in an additional fast-moving "amide-like" product (most probably because of acylation of the new reactive "arm" of the acceptor);
3. Aminogalactitol (AmG), a true isomer for AmS, was promptly recognized by the reverse-lipase catalyst (affording a dominant ester-like product); and
4. No product could be formed from disorbitilamine (the only assay where a marked drop of the reaction mixture solubility was visible).

Another feature worth mention was that of a parallel assay using the hydrochloride salt of aminosorbitol (AmS.HCl), which led to no product formation, too. This particular negative assay could indicate the need for a free *N:* electronic disposition at (C)-1-*N* for amide formation, but since no C(6)-ester was formed, most probably the negative effect resulted from a pH effect, a kinetic parameter to be further investigated.

An overall view of the theoretical course of the reaction for the basic model explored (AmS acceptor+C-9 donor) is shown in Fig. 4.

Gas-liquid chromatography characterization of the persilylated derivatives of reactants and products is shown in Fig. 5. As compared to the internal standard phenyl-D-galactopyranoside (R_T=9.64–9.71), the following resolutions were attained: pelargonic acid (=2.86), aminosorbitol (=7.39 >7.66; double peak profile probably arising from one or two derivatizations of both H at the amino group); main ester product (=11.25), and the easily crystallizable pearled product, the amide (=12.06). The amide products arising from the free acids octanoic (R_T=2.30) and heptanoic (=1.88), presented relatively shorter retention times (=10.58 and 10.04, respectively). Most of the reactants from a AmS+C-9 routine incubation were removed (free acid in the initial chloroform elution) or retained (AmS) in a silicic acid column. The sequential elution with methanol resulted in an enriched products preparation. Its GLC analysis (Fig. 5) showed a minor component with R_T=10.68 and it might correspond to the faint spot with middle Rf=0.55–0.57 and (+) reactions with both fluorescamine and rhodamine seen in the TLC plates (Figs. 1A and 1B;

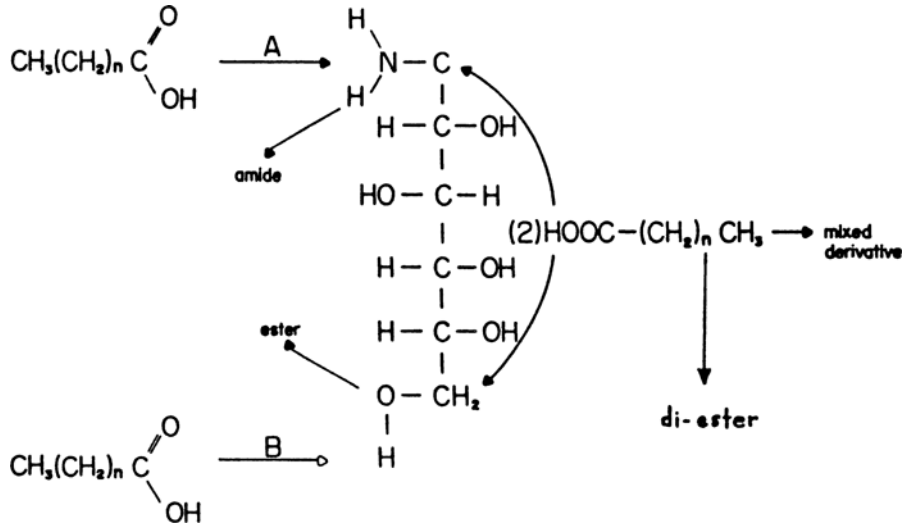

Fig. 4. Schedule for the enzymatic acylation of aminosorbitol.

lanes II to V). So it was suggestively interpreted as the diester of aminosorbitol, since it holds a free -NH_2 but moves ahead the main (mono)ester.

Because of the ease of crystallization experienced with the amide product (nonanoyl-aminosorbitol-amide), chromatographic resolution was also achieved using high performance liquid chromatography (Fig. 6). Fatty acids profile is shown in Fig. 6A and C-7 to C-9 members of the series (R_T=2.99, 5.26, and 10.37 min) are clearly distinguisible from the amide product (R_T=2.38 to 2.42) (Fig. 6C and 6D). No certainty could be derived for the ester(s) products (Figs. 6B and 6C), but probably they account for the peaks with higher R_{Ts}=3.55 and 6.41. Aminosorbitol comes off with the surplus solvent front (used to improve samples solubility in the run solvent), that is, in the void volume for the NovaPak C-18 column. Comparing the analyses for the whole incubation (Fig. 6B) with the methanol fraction from silicic column (Fig. 6C), the degree of amide and ester enrichment is clearly visible in the second sample. However, care should be exercized when manipulating the native products, since there were indications of amide content increase at the expense of ester product, in other words (C6)-O-acyl migration to the (C1)-*N*-acyl position. As an alternative HPLC procedure, the Fatty Acid column (Waters) could be used, which could result in sharper resolution for the free fatty acids (aminosorbitol still eluting in the void volume), but product peaks became too compressed between the solvent front and the first C-7 eluting fatty acid (results not shown).

Taking advantage of the crystalline form of the putative amide product (a pearled material displaying a peculiar crystalline array when recrystallized from aqueous pyridine or dioxane and microphotographed under polarized light; results not shown), a pure sample was dissolved in deuterated dimethyl sulfoxide and submitted to ^{13}-C nuclear magnetic

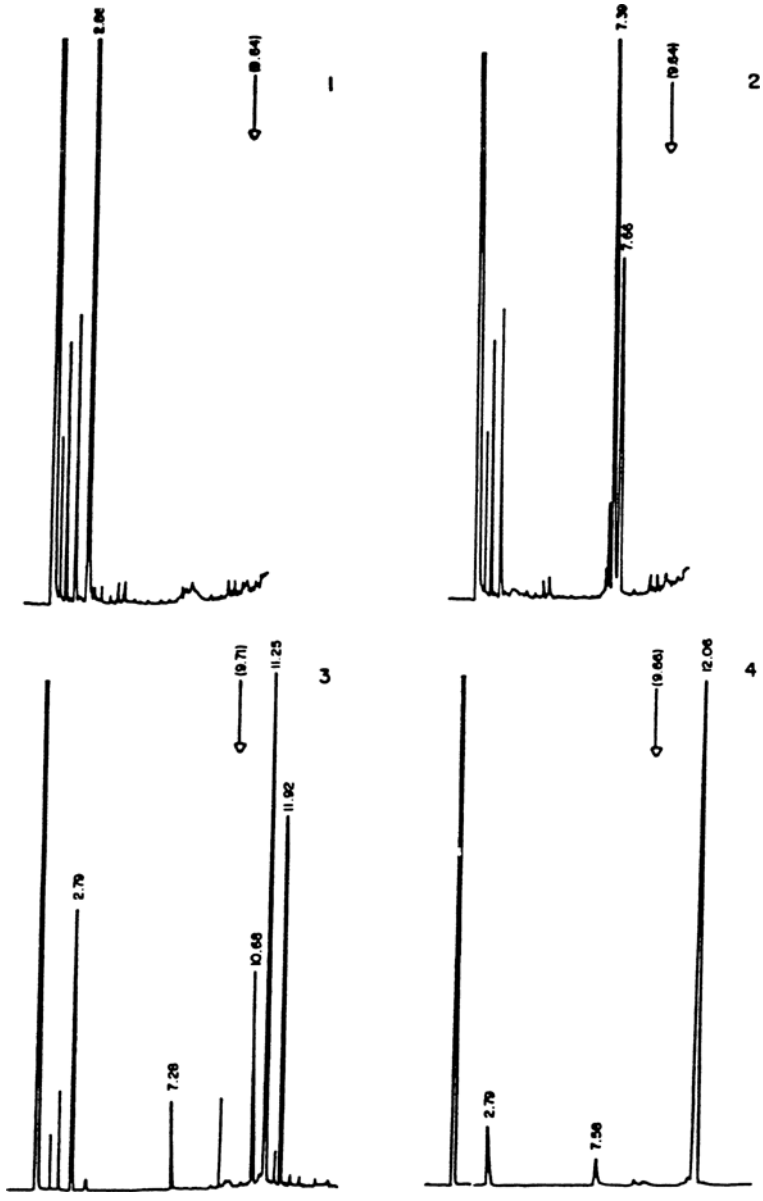

Fig. 5. Analysis of neolipid reactants and products by GLC. The HP-5 column (Hewlett-Packard; capillary, 25 m; 5% phenyl methyl-silicone; 0.2 mm diameter with a 0.33 m crosslinked film thickness) was programmed from 160°C (2 min) to 320°C at 16°C per min; nitrogen gas as carrier and a FID detector. Reactants or products were previously persilylated with a mixture of pyridine : BSTFA 1 : 1 at 70°C for 30 min.

Run 1 = pelargonic (C-9) acid standard
Run 2 = aminosorbitol standard
Run 3 = crude greasy product of incubation VI (sample M; TLC)
Run 4 = crystallized pearled product from incubation VI (sample P; TLC)
R_T = 9.64 to 9.71 refers to the retention time of the internal standard of persilylated phenyl-β-D-galactopyranoside.

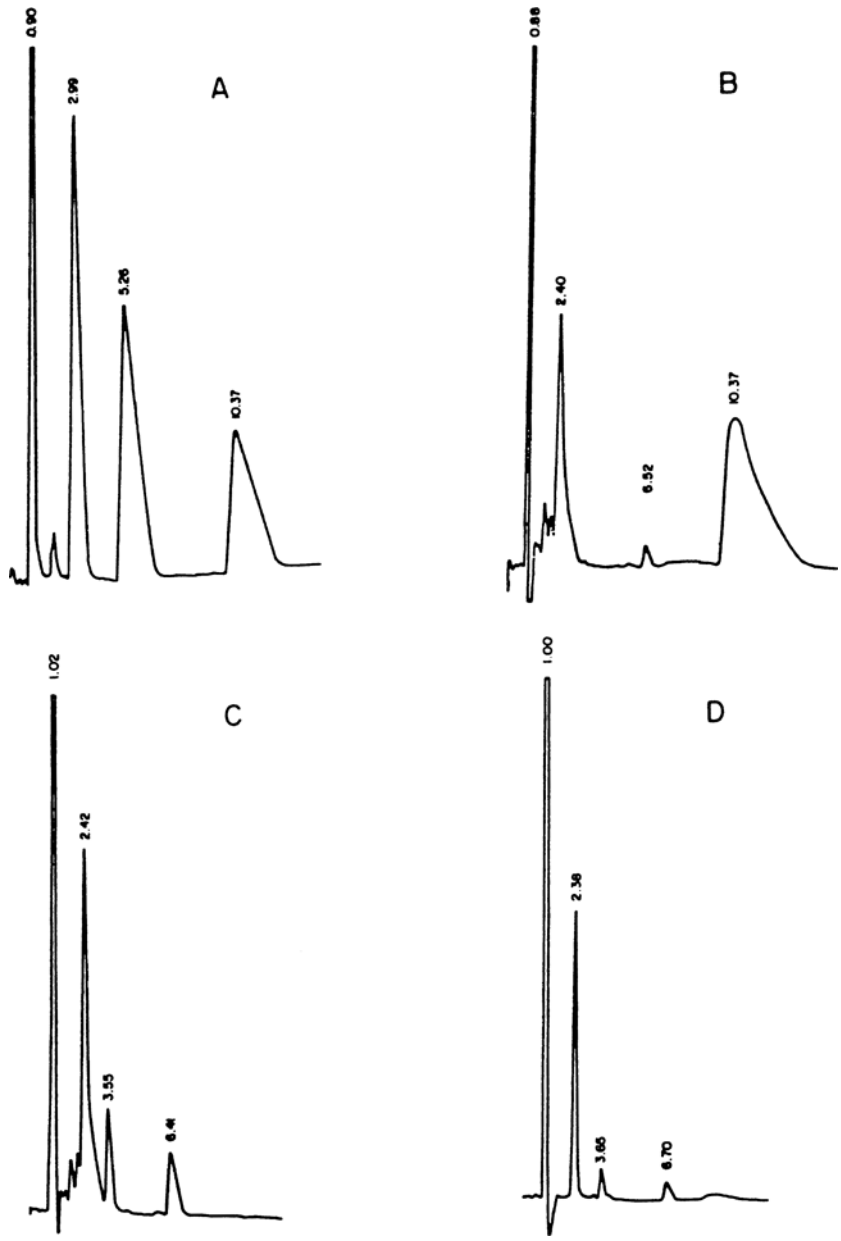

Fig. 6. Analysis of neolipid reactants and products by HPLC. A NovaPak C-18 column (8×0.3 cm) was equilibrated and isocratically eluted with the solvent mix indicated in M & M. Run time in abcissa and refraction index detector response at ordinate. A better sample solubility in the run solvent was ensured through the addition a surplus amount of methanol or dioxane (earliest peak in each run).

Run A = heptanoic, octanoic, and nonanoic (C-7, C-8, and C-9) fatty acid mixture
Run B = incubation VI (LPZ + AmS + C-9, in hexane at 35°C for 48 h)
Run C = fraction M of incubation VI (eluate with methanol from the silicic acid column)
Run D = pearled crystallined product from incubation VI.

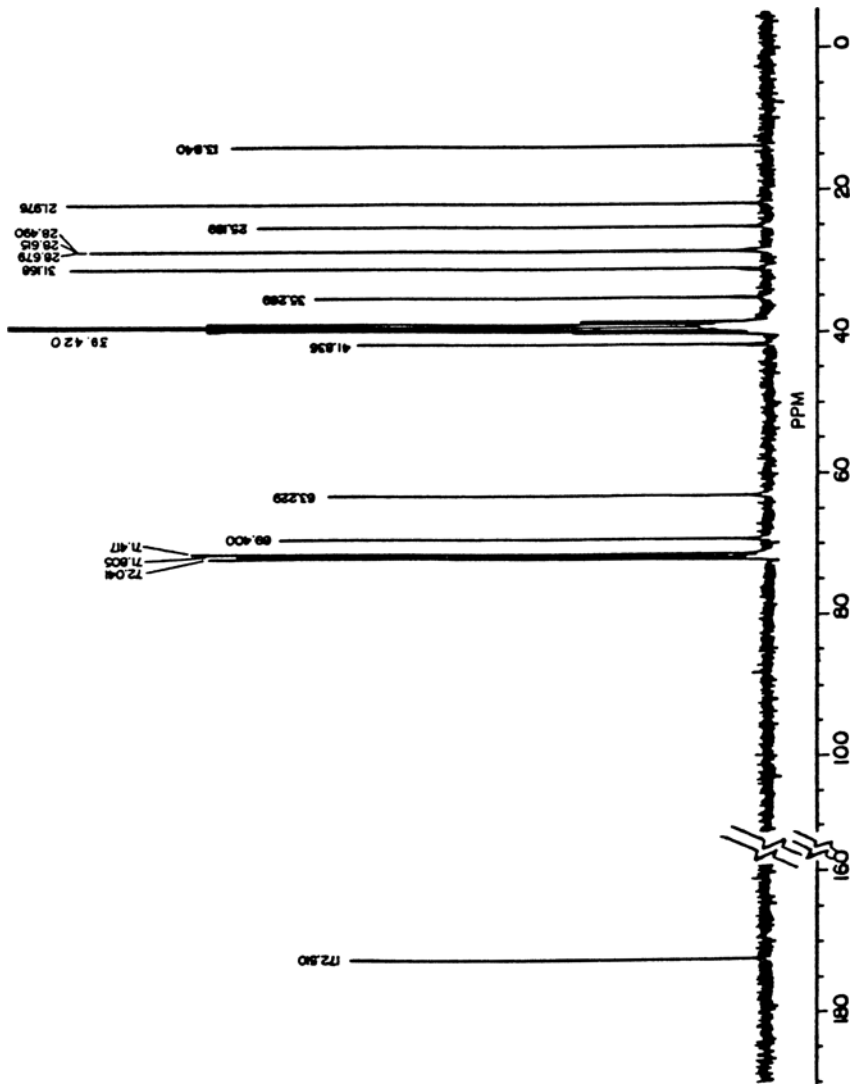

Fig. 7. Spectrum of ^{13}C-NMR of the crystalline product of aminosorbitol nonanoylation by reverse action of Lipozyme IM-20. A 60 mg sample of the pearled crystalline fraction generated during the manipulation of a standard incubation of LPZ+ AmS+ pelargonic (C-9) acid was dissolved in d_6-DMSO and analyzed in a model AC-300-P apparatus from Bruker (5 mm diameter tube) at 27°C. Chemical shifts (δ) are in ppm.

Table 1
Summary of the Major ^{13}C-NMR Signals Assignment (ppm)

C number	Aminosorbitol	Product	Nonanoic acid	Δ ppm (*)
C-6	63.62	63.23	—	0.39
C-5 to C-3	71.22–70.18	71.81–69.40	—	0.59–0.78
C-2	73.24	72.04	—	1.20
C-1 (NH_2)	**43.06**	**41.84**	—	1.22
C-1 (COOH)	—	**172.51**	**174.31**	1.80
C-2	—	35.27	33.55	1.72
C-3 to C-8	—	31.17–21.98	31.14–21.97	0.03–0.01
C-9 (CH_3)	—	13.84	13.78	0.06

(*) Normalization in the range of < 0.05 ppm was carried out by taking the middle signal of d_6-DMSO internal standard (39.42 to 39.39).

resonance (Fig. 7) in parallel runs to those obtained for the reactants in the same solvent and internal standard. The main chemical shifts (δ, ppm) were listed in Table 1. As can be seen, major spectral differences detected in the suggested amide product refer to C-1 and C-2 of both aminosorbitol and pelargonic acid, a clear indication for the generation of an amide linkage [1.22 and 1.20 ppm at C1-NH_2 and C2-(-CHOH-) from aminosorbitol moiety and 1.80 and 1.72 ppm at the C1-(-COOH) and C2-(CH_2-) from pelargonic acid moiety]. Accordingly, the C6-(-CH_2OH) primary alcoholic group of aminosorbitol remained almost unchanged comparing the free reagent and the respective moiety in the amide product (63.62–63.23 ppm). As a reinforcement for the structure ascribed to the crystallizable product, namely the nonanoyl amide of (C1-NH_2) aminosorbitol (Fig. 4, top), duplicate samples were separately boiled for 15 min in 0.1 and 4.0*M* TFA, considering the order of lability ester > amide toward strong acids. The product remained almost unchanged with the weaker acid, but it generated free aminosorbitol and pelargonic acid upon strong acid action as detected by TLC analysis. This simple test almost certainly excluded the possibility of the crystalline product being a double acylated compound, that is, bearing an additional ester at $C_{(6)}$-OH position of aminosorbitol beside the well characterized amide at $C_{(1)}$. Furthermore, the melting point obtained for this product was 147°C compared to 127°C for aminosorbitol (recall that pelargonic acid melts at 12.4°C). On the other hand, as a final analytical procedure, the main ester preparation was scaled up, but short-term AmS + C-9 incubations were kept. After several partitions between chloroform/water (for removal of residual free aminosorbitol in the aqueous layer) and preparative TLC (discarding the excess of pelargonic in the front zone), a grease was obtained. It was submitted to mass spectrometry in the direct inlet mode operation as an underivatized sample. Such a product should correspond to a (theoretical) mol wt = 321.

The main peaks [m/e (relative intensity)] observed under e.i. fragmentation were as follows: 43 (100), 73 (93), 30 (89), 57 (67), 60 (66), 86 (47), 158 (34), 102 (23), and (more important), 322 (4). The last one corresponded to M+1, and to the expected molecular ion. Peaks 60 and 73 were the usual fragments from a C-β (plus a C-γ H migration) and C-γ excisions of fatty acids (*11*). Furthermore, the peak 158 is incidentally coincident with the aminosorbitol moiety m.w. of the ester product (*12*).

CONCLUSIONS

As compared to the previously reported chemical synthesis of the named MEGA biological membrane solubilizers, or gluco-*N*-methyl-alkamides from methylglucamine (*13*), the immobilized form of a fungal lipase (*Mucor miehei* Lipozyme IM-20) proved to be a useful catalyst for the enzymatic synthesis of both ester and amide derivatives of the unmethylated acyl acceptor, 1-amino-1-deoxysorbitol or glucamine. A broad specificity was found for both acyl acceptors and donors. Apolar organic solvents favored the reaction rate and products yield, albeit their generation was also feasible in the absence of added solvent and with a higher load of acyl donor.

An optimization of the enzymatic coupling process seeking for improved yields deserves further attention since the ester product, at least, owing to its stable foam-forming ability and satisfactory water solubility, may find technological application as a surfactant. Also to be clarified remains the role of the whole conjugate(s), recalling the inhibitory property of its aminosorbitol moiety.

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