The Catalytic Activity of Lipases Toward Hydroxy Fatty Acids—A Review

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ABSTRACT: Hydroxy fatty acids, derived from several natural and synthetic sources, have many applications. Lipases have been employed to catalyze reactions involving hydroxy acids to narrowly shape the product distribution *via* their regio- and stereoselectivities. This manuscript reviews the action of lipase on hydroxy acids and their derivatives. The formation of estolides or lactones by lipase-catalyzed reactions depends strongly on the position of the hydroxyl moiety on the hydroxy acyl group and slightly on the hydroxy acid chainlength and concentration. *Pseudomonas* sp. and porcine pancreatic lipases are the most useful for catalyzing formation of optically pure lactones, while lipases lacking positional selectivity catalyze estolide formation best. The product distribution of lipase-catalyzed esterification between hydroxy- and nonhydroxy-acyl groups is strongly dependent on the lipase type. Lipase-catalyzed reactions between hydroxy acids and alcohols yield hydroxy esters, not estolides, as the major product.
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KEY WORDS: Enantioselectivity, estolides, hydroxy fatty acids, lactones, lipases.

Hydroxy fatty acids (HFA) are multifunctional molecules that have a variety of applications. HFA and their derivatives are used in cosmetics, paints and coatings, lubricants, and the food industry. They are useful chemical intermediates in the synthesis of fine chemicals and pharmaceuticals, particularly when they are optically pure (except for ω-HFA, all HFA contain at least one chiral center—at the hydroxyl-bearing carbon). HFA are derived from a variety of natural sources, including plant seed oils, glycosides, microorganisms, epicuticular waxes of coniferous trees, plant cutin, tall oil, and cork. Some of these HFA are listed in Table 1. A more complete listing can be obtained elsewhere (1).

For many of the natural sources, HFA are found as parts of estolides, which are oligiomeric molecules that consist of hydroxy acyl groups bonded together *via* ester bonds between the hydroxy side-chain moiety of one acyl group and the –COO functionality of another. Sources of naturally occurring estolides are reviewed elsewhere (3). Estolides have potential uses as lubricants, cosmetics additives, and printing ink dispersants.

HFA are also encountered in nature as cyclic esters known as lactones (e.g., in homogenized milk, fruit, tobacco, and insect pheromones). A single HFA forms a lactone in the solution under acidic conditions *via* formation of an intramolecular ester bond (4). An oligomeric HFA chain (i.e., an estolide), when intrasterified at its -OH and -COO termini, forms a large

TABLE 1
Selected Hydroxy Fatty Acids (HFA) and Their Sources

Common HFA name	Structure	Source
2-Hydroxy lauric	$12{:}0\text{—}OH^{2}$	*Azotobacter. agilis*
3-Hydroxy oleic	$18{:}1^{9c}\text{—}OH^{3}$	*Alcaligenes* sp.[a]
Ricinoleic	$R\text{-}18{:}1^{9c}\text{—}OH^{12}$	*Ricinus comminus* (Castor) seed
C_{18} ω-HFA	$18{:}0\text{—}OH^{18}$	Epicuticular wax (Pine trees)
Densipolic	$18{:}2^{9c,15c}\text{—}OH^{12}$	*Lesquerella densipila* seed
Dimorphecolic	$18{:}2^{10t,12t}\text{—}OH^{9}$	*Dimorphotheca sinatua* seed
α-Kamlolenic	$18{:}3^{9c,11t,13t}\text{—}OH^{18}$	*Mallatus phillipinensis* seed
Lesquerolic	$R\text{-}20{:}1^{11c}\text{—}OH^{14}$	*L. fendleri* seed
15-Hydroxy eicosaenoic	$S\text{-}20{:}5^{5c,8c,11c,13t,17c}\text{—}OH^{15}$	*Laminaria* sp. (Brown algae)
Phellonic	$22{:}0\text{—}OH^{13}$	Cork
Ipurolic	$14{:}0\text{—}OH^{3,11}$	*Ipomoea purga* (plant tubers)
Dihydroxyhexadecanoic	$16{:}0\text{—}OH^{9,16}$	Coffee or papaya (Cutin)
Ustilic	$16{:}0\text{—}OH^{15,16}$	*Ustilago zeae* (mold)
(+)-Threo-9,10,18-trihydroxyoctadecenoic	$18{:}1^{12c}\text{—}OH^{9,10,18}$	*Chamaepeuce afra* seed

[a]Fermentation product. See Reference 2. *c, cis; t, trans.*

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cyclic molecule known as a polyolide. Lactones/polyolides are used in perfumes, as flavor components in food, as plant growth regulators, and as chemical intermediates for the synthesis of biologically active materials. Most naturally occurring lactones are optically active γ- and δ-lactones. HFA, estolides, and lactones can also be derived in the laboratory by a variety of synthetic methods.

Lipases (EC 3.1.1.3) have proven to be valuable catalysts for reactions involving HFA and their derivatives, particularly synthetic (esterification) reactions. In particular, lipases have been shown to catalyze a wide variety of reactions, often with good stereoselectivity. This is relevant because HFA contain chiral centers. Additionally, lipase-catalyzed reactions are less prone to side reactions than are reactions conducted with conventional catalysts. Furthermore, lipase-catalyzed reactions operate at mild reaction conditions, which prevents degradation of delicate starting materials and promotes lower operating costs. The main objective of this review is to examine the types of reactions involving HFA that lipases can catalyze and the biocatalytic specificity of lipases toward hydroxy acids.

ACTIVITY OF LIPASES TOWARD HYDROXY ACIDS AND THEIR DERIVATIVES

Specificity of lipases toward HFA molecular structure (1-substrate reactions). The location of the hydroxyl group on the fatty acid chain has a pronounced influence on whether estolides or lactones are formed when an HFA or hydroxy acid ester (HAE) is exposed to the biocatalytic action of lipase (Table 2). When the hydroxyl group is located at carbon number 3 (C_3), i.e., in the β-position, only estolides are formed. When the hydroxyl group is at C_4 (γ) or C_5 (δ), lactones are preferably synthesized (γ- and δ-lactones are the most commonly encountered lactones in nature). An exception to this rule is 5-hydroxy pentanoic acid, which under most conditions yields estolide (Table 2) (5). This is because 5-hydroxy pentanoic acid is also an ω-HFA (see below).

Omega- and (ω1)-HFA, when present at low concentrations, yield polyolides for short-chain HFA (C_{10}–C_{14}), while long-chain HFA yield monoacyl ω-lactones (14–18). Omega HFA, when present at larger concentrations, yield estolides. Recent reports demonstrated that pancreatic and *Pseudomonas fluorescens* lipase utilization of 5-hydroxy pentanoic acid and 6-hydroxy hexanoic acid (or equivalently, their lactones) yields estolides with molecular weights as high as 7000–12,000 after a 10–30-d reaction period (10,13). The degree of polymerization and rate of reaction increased with reaction temperature (13,27). In addition, ω-C_{10} and ω-C_{11} hydroxy acids (or their lactones) yielded estolides with molecular weights from 20,000–35,000 after a few days of reaction time (26,27,35). The degree of polymerization attained with lipases for these substrates is much greater than that achieved with nonenzymatic methods (36). O'Hagan and Zaidi (35) report that oligomerization of C_{16} ω-HFA failed. However, Uyama *et al.* (27) report that estolides of molecular weight 1000–6400 were synthesized from the lactone of C_{15} ω-HFA at a slightly lower reaction rate than from C_{11} ω-HFA lactone, but at a much greater rate than from C_6 ω-HFA lactone. These trends are opposite to that obtained with nonenzymatic catalysts, where the degree of polymerization decreased as the chainlength of the ω-HFA lactone increased due to a decrease in the ring strain (27).

TABLE 2
Effect of Chainlength and Hydroxyl Position of Straight-Chain Hydroxy Acyl Groups on the Product of Lipase-Catalyzed Esterification (1-substrate reactions)

Carbon length	-OH position	Product	References
3	3(β)	Estolide	5
4–8	4(γ)	γ-Lactone	5–9
5	5(γ,δ)	Estolide	5,10
6–16	5(δ)	δ-Lactone	5,9,11,12
6	6(ε,ω)	Estolide	5,10,13
8,11	ω1	ω-Lactone/polyolides	14,15
10–20	ω	ω-Lactone/polyolides[a]	6,14–24
		Estolide[b]	23,25–27
13–15	ω6	Lactone and estolide	28
18	12	Estolide	25,29–33
20	14	Estolide	34

[a]Low (less than 10 mM) initial concentration of hydroxy acyl substrate.
[b]High initial concentration of hydroxy acyl substrate.

Two examples are included in Table 2 for long-chain HFA that contain hydroxyl moieties at a carbon atom in the center of the molecule: ricinoleic and lesquerolic acids (last two entries). In both cases, estolide was the major product, with no other products detected or reported. The investigations that employed ricinolenic acid and 12-hydroxy stearic acid yielded penta-acyl estolides (tetraestolides) as the largest molecular weight species, with the average number of acyl groups per estolide falling between two and three. Estolide formation proceeded readily whether the medium consisted of large quantities of water or was nearly anhydrous, or whether the HFA was in free fatty acid form or part of triglycerides (31–34). The lower degree of polymerization achieved with ricinolenic or lesquerolic acid in comparison to ω-HFA of carbon chainlength 6–15 (see above) is intriguing.

Reactions involving ricinolenic acid yielded entirely estolide (Table 2) with two exceptions: Yamada *et al.* (18) and Lobell and Schneider (28) produced lactone when the fatty (ricinolenic, ricinelaidic, or 12-hydroxystearic) acyl group concentration was ≤5 mM. The yield of lactone reported by Yamada *et al.* (18) from free ricinoleic acid was only 14–20%. Lobell and Schneider (28) reported lactone yields of *ca.* 70%, with no estolide produced when the hydroxy acyl groups were in the form of vinyl esters, but the yield and selectivity toward lactone decreased dramatically when the acyl groups were in free fatty acid or methyl ester form. They also found that hydroxy acid vinyl esters for a series of (ω6) hydroxy acyl groups improved yield and lactone production, presumably due to the irreversibility imposed by the vinyl alcohol leaving group, which tautomerizes into an aldehyde (28).

The initial hydroxy acid substrate concentration is also a major factor in dictating the product distribution. Moreover,

an HFA's own -OH group will compete with the -OH groups of other HFA for esterification of its carbonyl group. Thus, lactone formation is generally enhanced at lower substrate concentrations. This trend is exhibited for ω-HFA. Moreover, lactones (and polyolides) are the major products formed when ω-HFA are present at concentrations ≤1 mM (10,15–17,21,22). Using 6-hydroxy hexanoic acid as substrate, Knani *et al.* (10) found that lactones/polyolides were produced at 46.7% when the substrate concentration was at 67 mM, but was much less at higher concentrations. In addition, 5-hydroxy pentanoic acid, when at 165 mM, yielded mostly estolide (e.g., Table 2); however, 31.3 mM substrate yielded δ-lactone as product (5). Several reports demonstrate that the yield of lactone decreased as the ω-HFA substrate concentration was increased (17,21,22,24). Moreover, estolides are produced almost exclusively from ω-HFA when the latter is present at 22.5 mM in organic solvent or in bulk (i.e., in the absence of organic solvent) (23,25,35).

For γ- and δ-lactonization, substrate concentration is a less significant factor; moreover, γ- and δ-hydroxy acyl group concentrations between 3.5 and 250 mM yielded lactone as the major product (5,7,9,37–39).

The product concentration is also an important factor. Robinson *et al.* (22) have demonstrated that the presence of hexadecanolide product is inhibitory to the lactonization of C_{16} ω-HFA.

The research group of G.D. Reed in Norwich, United Kingdom, have conducted ω-lactonization reactions in water–oil microemulsion systems (19,24). They found that the presence of surfactant significantly increased the solubility of ω-HFA in organic solvent (24). Yields of monoolide between 50–60% were achieved with several lipases in Aerosol-OT and CTAB-based microemulsion systems for 16-hydroxy hexadecanoic acid or its methyl ester at concentrations below 10 mM (19,24). Polyolides were not produced to any significant extent (24).

An increase in temperature but not beyond the point of thermoinstability of the biocatalyst has been shown to increase the yield of lactone, particularly for ω-hydroxy acyl groups (10,18,21,37). This may be related to the improved solubility of hydroxy acids in organic solvents at higher temperatures. In addition, the rate and extent of estolide synthesis from ω-HFA and their lactones were increased at elevated temperatures (13,26,27). However, for γ- and δ-lactonization, room temperature proved to be quite adequate (5,7,9,11,37–39).

The water content is also an important parameter. Robinson *et al.* (22) demonstrated that the addition of water decreased the rate and amount of lactone formation from C_{16} ω-HFA.

The effect of substitution on the aliphatic chain of HFA on the success if lipase-catalyzed lactonization has been examined. Substitution at the hydroxy-containing carbon (γ- and δ-) or at carbon molecules between C_6 and the ω-carbon did not prevent lactonization (7,37,38). In addition, the presence of a hydroxyl group at the β-carbon of a δ-HFA did not inhibit lactonization (12,39). However, inclusion of a methyl group at the α-carbon of a δ-HFA significantly reduced the rate, extent, and degree of stereoselectivity of lipase-catalyzed lactonization (7).

Several lipases have been screened for their ability to catalyze reactions with the -OH moiety of various HFA. Results are summarized in Table 3. The table demonstrates that *Pseudomonas* sp. lipases are generally the most active lipases toward HFA. The lipases that successfully catalyzed reactions with α- and β-HFA did so with quite high enantioselectivities, but in the case of α-HAE, enantiomeric excesses, or *ee*'s (defined in Ref. 53) were sensitive to the alcohol moiety esterified to the hydroxy acyl group's carbonyl (40–42). Both *Pseudomonas* sp. lipase and porcine pancreatic lipase (PPL) were quite successful in catalyzing enantioselective lactonization of γ- and δ-HAE, with the former lipase preferably synthesizing the R-enantiomer and the latter the S-enantiomer. However, when PPL acted upon a γ-HAE with a benzyl group on the γ-carbon, the R-enantiomer was preferably lactonized (7). HFA were not used as substrate to avoid spontaneous lactonization, which would lower the reaction's enantioselectivity. Huffer and Shrier (9) found that even γ- and δ-hydroxy acid methyl- and ethyl-esters spontaneously lactonized to a slight degree. They also discovered hydroxy acid propyl- and isopropyl-esters did not lactonize spontaneously, but that only the former was a suitable substrate for PPL (9). It is reported that even heat-denatured *Pseudomonas* lipase can catalyze lactonization from a δ-HAE, but with no enantioselectivity (11). γ-Lactonization of hydroxy acyl groups with carbon length of 6–8 yielded high values of *ee* for both product (ee_p) and remaining substrate (ee_s), but ee_p declined when the chainlength was increased from 8 (9). Yamada *et al.* (11) found that the enantioselectivity of *Pseudomonas* lipase toward δ-lactonization improved when the lipase was immobilized, and Triton surfactant was added. Values of *ee* for δ-lactonization were generally smaller than those for γ-lactonization; however, substitutions in the δ-acyl chains enhanced enantioselectivity and yielded high values of ee_p (14,18). As was true for *Pseudomonas* sp. lipase-catalyzed γ-lactonization, the R-configuration of these lactones was preferentially formed. However, the estolide by-product synthesized during 12-hydroxy octadecanoic acid lactonization was racemic (18).

A greater number of lipases was able to catalyze lactonization of ω-hydroxy acyl groups compared to γ- and δ-hydroxy acyl groups (Table 3). Reactions involving estolide formation from ricinoleic acid or hydrolysis of estolides were catalyzed successfully by "random lipases," meaning those that lack 1,3-positional selectivity (e.g., *Candida rugosa, Chromobacterium viscosum, Pseudomonas* sp., and *Geotrichum candidum*), and unsuccessfully by 1,3-specific lipases (e.g., *Rhizopus* sp., *Rhizomucor miehei*, PPL) (Table 3). This may be related to the inability of several 1,3-selective lipases to attack secondary alcohols (54). However, the ability of PPL to catalyze γ- or δ-lactonization apparently violates this rule. To explain, it has been reported that certain PPL preparations can utilize secondary-OH groups, indicating that PPL has less 1,3- positional selectivity (54,55).

Reactions between HFA and nonhydroxy acyl groups. Engel *et al.*(56) reacted various HAE of acyl chainlength 6 with octanoic acid in the presence of *C. rugosa* lipase

TABLE 3
The Activity of Various Lipase Types Toward the -OH Moiety of Hydroxy Acyl Groups and Toward Hydrolysis and Alcoholysis of Estolides

Substrate	Successful lipases[a]	Unsuccessful lipases	References
α-HFA[b]	*Pseudmonas* sp., *C. viscosum*	PPL, *C. rugosa*	40
β-HFA[b]	*Pseudomonas* sp., *C. rugosa*, PPL[c]		41,42
γ-HFA/HAE	PPL, *Pseudomonas* sp.	*A. niger, C. rugosa*[d], *R. miehei, Rhizopus* sp., wheat germ	7,9,37,38,43,44
δ-HFA/HAE	PPL, *Pseudomonas* sp.		9,11,12,39,44
ω-HFA/HAE	PPL, *Pseudomonas* sp., *C. viscosum, C. antarctica, Alcaligenes, A. niger*	*Rhizopus* sp.[e], *C. lipolytica, G. candidum, P. cyclopium,* wheat germ, *C. rugosa*[e]	6,13–27,35
Ricinoleic acid	*G. candidum, C. rugosa, C. viscosum, Pseudomonas* sp.	*Rhizopus* sp., PPL, *R. miehei*	25,29–33,45,46
Estolide[f]	*Pseudomonas* sp., *G. candidum*, PPL[g], *C. rugosa, Rhizopus* sp.[h]		47–52

[a]Full names of lipase types: *Aspergillus niger, Candida lipolytica, Candida rugosa, Chromobacterium viscosum, Geotrichum candidum, Penicillium cyclopium, Rhizomucor miehei;* PPL, porcine pancreatic lipase; HAE, hydroxy acid ester. See Table 1 for other abbreviation.

[b]Reaction was between HFA or its ester and a short-chain acyl group activated ester.

[c]Yield of product with PPL as biocatalyst was lower (Ref. 41).

[d]Reference 43 indicates *C. rugosa* lipase synthesizes lactone formation, but at poor yields.

[e]References 6, 14, 25 indicate that *R. miehei* and *C. rugosa* lipases yield lactones and other products at small yields. References 15, 16, 24 suggest that *C. rugosa* lipase is a poor biocatalyst for lactone/polyolide formation. References 13, 27 demonstrate for estolide synthesis from lactones of ω-HFA that *C. rugosa* lipase "B" from Cosmo Bio Corp. (Tokyo, Japan) was successful, whereas *C. rugosa* lipase "AY" from Amano (Troy, VA) was not successful.

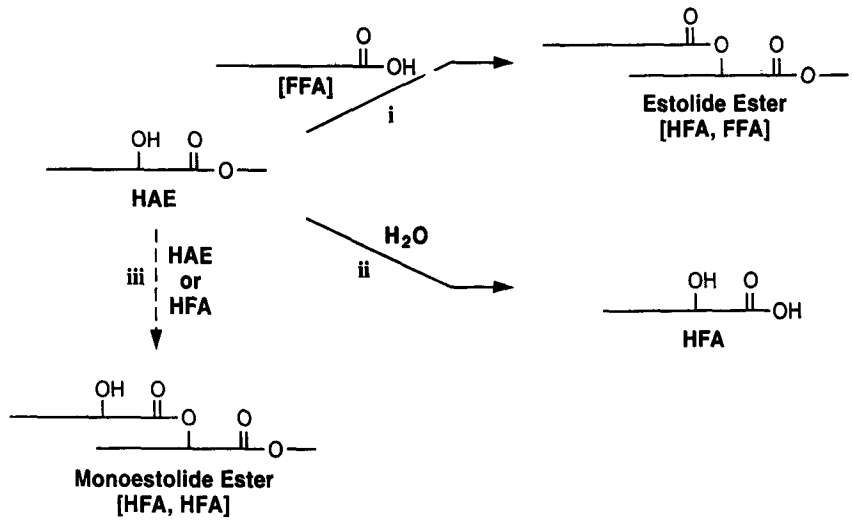
[f]Estolide hydrolysis.

[g]Extent of hydrolysis with PPL is less than that of other enzymes (Ref. 50).

[h]*Rhizopus* sp. lipase was successful in hydrolyzing estolides of ω-HFA only (Refs. 51,52).

(Scheme 1). They found that when the -OH group is at the α-position, only small amounts of estolide were synthesized (reaction step i of Scheme 1), but a large amount of free HFA was formed (step ii). Estolide formation between two hydroxy acyl groups (step iii) was not reported and probably did not occur (see below). Both reaction products had high values of *ee*. When the -OH group was at the β- or δ-carbon, estolide formation occurred much more rapidly than hydrolysis, but values of *ee* were low (56). In addition, if these reactions were allowed to proceed to equilibrium, nearly equimolar amounts of free HFA and estolide were produced. In other experimental work, α- and β-hydroxy acid esters were successfully esterified with short-chain free fatty acids when the latter were present as activated esters (e.g., vinyl acetate) (40–42). Hayes and Kleiman (34) performed a series of reactions between lesquerolic and octadecanoic acids. They found that 1,3-selective lipases [with the exception of *Aspergillus niger* lipase, which possesses weaker positional selectivity (55)] did not catalyze esterification, while *A. niger* and *Penicillium cyclopium* lipases catalyzed only a small amount of estolide formation (Table 4). The product distribution also varied with lipase type. Even though lesquerolic acid was present in stoichiometric excess relative to octadecanoic acid, *ca.* 85% of the product was a monoestolide that contained one lesquerolic acyl group and one C_{18} group when *C. rugosa, G. candidum*, or *P. cyclopium* lipases were employed. When *Pseudomonas* sp. or *A. niger* lipase were employed, monoestolide that contained two hydroxy acyl groups and diestolide were produced at more significant amounts. Thus, lipases have a wide variety of specificities toward reactions between hydroxy and nonhydroxy acids.

Reactions between hydroxy acids (or estolides) and alcohols. As mentioned above, most 1,3-specific lipases cannot act upon HFA. This has been taken advantage of in the esterification of HFA and alcohol so that estolide or lactone formation is avoided. For example, *R. miehei* lipase has been employed in the esterification of several different HFA (46,57,65) and free estolide (34) However, even random lipases catalyze more favorably ester formation over estolide/lactone formation. For example, *C. viscosum* lipase catalyzed esterification between 16-hydroxy hexadecanoic acid and 1-hexanol without lactone or diolide formation oc-



SCHEME 1

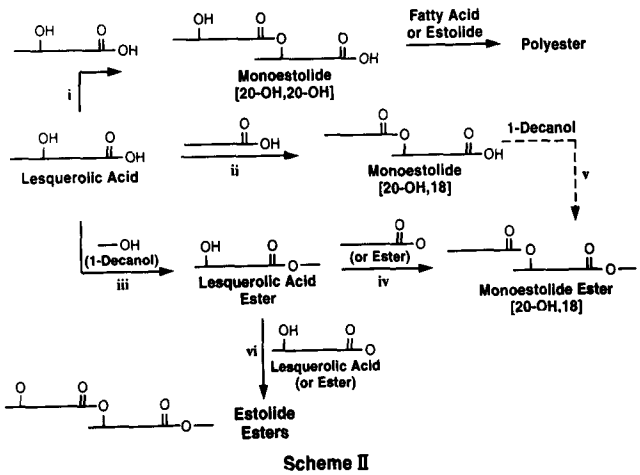
curring (62). In addition, *C. rugosa* lipase catalyzed the synthesis of dodecyl 17-hydroxy stearate, butyl 3-hydroxy proprionate, and butyl 2-hydroxy caprylate without estolide or lactone being formed (57,61,66). Similarly, Bevinakatti *et al.* (50) reported that *C. rugosa* lipase-catalyzed butanolysis of estolide ester does not break the estolide bond. Hayes and Kleiman (34) reacted nearly equimolar amounts of HFA, octadecanoic acid, and 1-decanol in the presence of each *C. rugosa* and *Pseudomonas* sp. lipases (Scheme 2). The former lipase yielded only fatty acid ester as product (reaction step iii of Scheme 2). The latter enzyme yielded fatty acid esters quickly and in large amounts. Slowly, the fatty acid ester content decreased while estolide ester concentration increased. Because no free estolide was ever detected (i.e., steps ii and v of Scheme 2 did not occur), *Pseudomonas* sp. lipase-catalyzed acidolysis of HAE by C_{18}-fatty acid ester (step iv) did occur.

TABLE 4
Estolide Formation from a Lesquerolic Acid/Octadecenoic Acid Mixture[a]

Lipase type[b]	Esterification[c] %	Product distribution Monoestolide [20-OH,18]	Monoestolide [20-OH,20-OH]	Diestolide
C. rugosa	41.3	83.9	13.5	2.6
G. candidum	45.2	80.6	14.4	5.0
P. cyclopium	13.7	83.4	16.6	0.0
A. niger	12.3	65.7	30.2	4.1
Pseudomonas sp.	62.8	51.1	29.0	12.5
1,3-Selective lipases[d]	0.0			

[a]Reaction medium contained 2.5 mL each fatty acid substrate (*ca.* 82% C_{20}-hydroxy acid and 15.5% C_{18} unsaturated acids) and isoctane and 5.0 mL 50 mM phosphate buffer (pH = 6.8) that contained lipase. Reactions were conducted at 22°C under magnetic stirring (300 rev min^{-1}). From Reference 34.
[b]Full names of lipase types are listed in Table 3.
[c]After 2–3 d reaction time.
[d]*R. miehei* and *R. arrhizus* lipases: See Table 3 for lipase types abbreviations.

Estolide hydrolysis reactions. Hayes and Kleiman (34) examined lipase-catalyzed hydrolysis of estolides derived from oleic acid. The estolides contained no free hydroxyl groups, and the hydroxyl acyl groups within these estolides had their -OH moieties located between C_7 and C_{11}. Based on a variety of -OH positions within HFA that lipases can utilize (Table 2), lipases should be able to act upon this substrate. For estolide hydrolysis, catalyzed by either *C. rugosa* or *Pseudomonas* sp. lipases, the extent of hydrolysis was small: *ca* 10%. This held true in all types of medium examined: organic solvent-based medium, water-in-oil microemulsions (reverse micelles), and aqueous-lipophilic organic solvent biphasic medium, the latter of which had a high water content. Similarly, high water content did not limit ricinoleic acid estolide formation in biphasic medium (25). Hence, estolide formation is a reaction that is strongly driven in the forward direction. However, in aqueous medium, lipolysis of water-soluble estolides has been conducted successfully at high yields (47–49). In addition, poly-ε-caprolactone (poly-C_6ω-



SCHEME 2

HFA lactone) was successfully hydrolyzed in aqueous medium when emulsified with surfactant(51,52). Poly-$C_6\omega$-HFA lactone that had more amorphous structure was more readily hydrolyzed than that containing highly crystalline structure (52). Several different random lipases were successful in hydrolyzing estolides (Table 3), and reaction enantioselectivity was high. In addition, Lu *et al.* (63) successfully hydrolyzed estolide in a polar organic solvent, 1-butanol.

In conclusion, HFA can be successfully employed as substrates for various reactions catalyzed by lipases. Lipases are valuable tools for synthesizing optically pure estolides and lactones and for separating enantiomeric mixtures of HFA. Lipase-catalyzed HFA reactions will continue to be used as the demand for optically-pure molecules and biocompatible polymers increases in the pharmaceutical industry and elsewhere.

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