Lipolytic Synthesis of Optically Active 1,2-Dibutyryl-*sn*-Glycerol. Identification of Diglyceride by Solvent-Dependent Specific Rotation

Charmian J. O'Connor*, Douglas T. Lai, and Richard H. Barton

Department of Chemistry, The University of Auckland, Auckland, New Zealand

ABSTRACT: The objective was to determine whether the initial pregastric lipase catalyzed hydrolysis of a triacylglycerol to 1,2(2,3)-diacylglycerol was a consequence of *sn*-specific hydrolysis. The identity of the reaction products for the enzyme-assisted hydrolysis and uncatalyzed acyl-transfer reaction sequence of tributyrylglycerol was assigned by ^{13}C nuclear magnetic resonance. The optical activity of the product 1,2-dibutyryl-*sn*-glycerol (yield >50%, pH 6.5, 35°C, 13 min) was solvent dependent, being –2.92° (c ~1.3, $CHCl_3$) and +3.32° (c ~1.2, pyridine), and confirmation of *sn*-3 specificity by pregastric lipase was obtained.

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KEY WORDS: Acyl transfer reactions, 1,2-dibutyryl-*sn*-glycerol, lipid hydrolysis, optical rotation, pregastric lipase, racemization, stereoselective hydrolysis.

Mammalian pregastric lipases are secreted from the pharyngeal and epiglottal region during suckling or swallowing (1). In the neonate, their natural substrates are milk lipids and they show reactive preference for short-chain fatty acids, which are sequestered in the *sn*-3 position in milk fat (2). Under physiological concentrations of pregastric lipase, hydrolysis of triacylglycerols is initially only by monoacyl hydrolysis to form 1,2(2,3)-diacylglcerols (3).

We have previously shown (4) that the sequence **1** → **2** → **3** (Scheme 1) takes place by a rapid enzyme-assisted hydrolysis (k_1) followed by a much slower noncatalyzed acyl transfer reaction (k_4). This investigation confirms that **2** appears as the result of stereospecific *sn*-3 hydrolysis of **1**.

EXPERIMENTAL PROCEDURES

Example I employed 483 mg industrial-grade lamb pregastric lipase, 1.75 mmol **1** in 40 mL bis-tris-propane buffer, pH 7.00, 35°C. After 13 min, a 30-mL aliquot was extracted with $CHCl_3$, and after measurement of its optical rotation, its mass composition (Table 1) was determined by ^{13}C nuclear magnetic resonance (NMR) at 400 mHz. The specific rotation was then calculated after applying the appropriate mass correction to the extracted sample, to yield $[\alpha]_D^{20} = -2.92°$ (c ~1.3, $CHCl_3$). After removal of the $CHCl_3$, the sample was redissolved in pyridine and the specific rotation measured to yield $[\alpha]_D^{20} = +3.32°$ (c ~1.2, pyridine), where c is percentage wt/vol concentration in solvent (g/100 mL).

Example II employed 1080 mg industrial-grade enzyme and 2.24 mmol **1** under the same conditions as for Example I. After 15 min, a 30-mL aliquot was removed and the specific rotations were determined in the two solvents in reverse order to yield $[\alpha]_D^{20} = +2.81°$ (c ~1.4, pyridine) and –2.15° (c ~1.2, $CHCl_3$).

RESULTS AND DISCUSSION

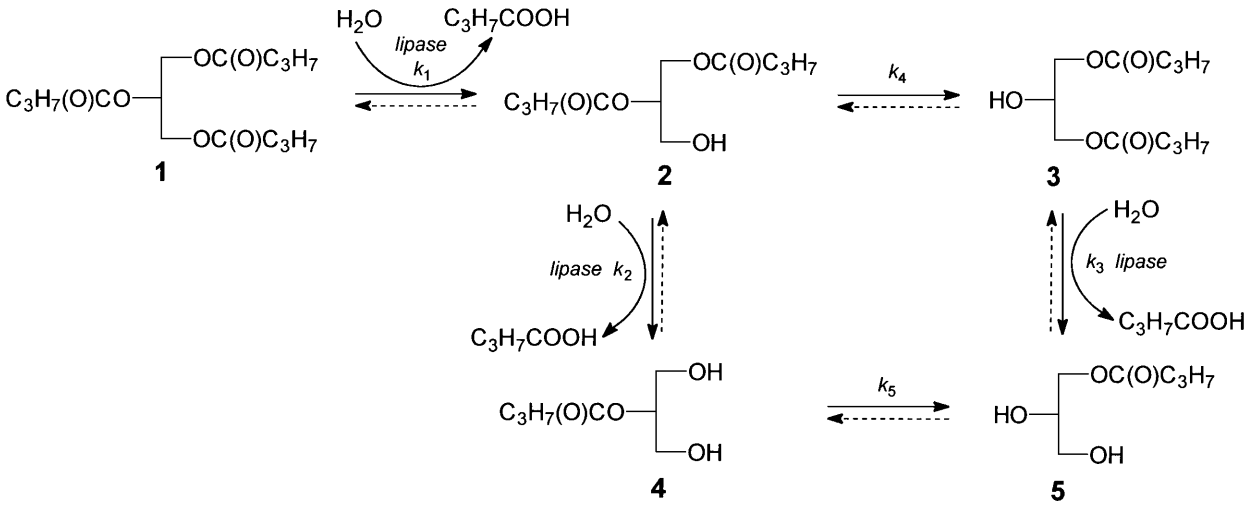
By altering the relative concentrations of lipase:**1** and analyzing the composition of the product-mixture by ^{13}C NMR, we have identified the presence of **2**–**5** (Scheme 1) during the lipase-assisted hydrolysis of **1**. Typical composition mixtures are given in Table 1. Compound **2** predominates over **3** in fast reactions (the concentration of **1** remaining is small), and under these rapid catalyzed rate conditions the preferred route for production of **5** is *via* acyl transfer from **4**, which in turn is produced by enzyme-assisted hydrolysis of **2**. Of all the species present in a product mix, only **2** and **5** are potentially optically active, and measured activity will depend on the extent to which each is truly *sn*-pure, i.e., *sn*-1,2 and not also *sn*-2,3, which would yield d(+) and l(–) specific rotations, respectively. ^{13}C NMR spectroscopy cannot distinguish between these enantiomers. Compound **5** will be optically inactive if it is produced *via* the uncatalyzed (random) acyl migration step (k_5). The reaction and extraction procedures described were carried out so as to minimize the initial extent of acyl transfer, k_4, and to retain optical activity.

The value of $[\alpha]_D^{20}$ in $CHCl_3$ (–2.92°) (Example I) was in conflict with the value (5) for α,β-dibutyrin ($[\alpha]_D^{20} = +1.7°$, c

TABLE 1
Mass Percentage Composition of Products of Enzyme Catalyzed Hydrolysis of 1

Example	Reaction time (min)	Mass %				
		1	**2**	**3**	**4**	**5**
I	13	53.7	36.8	7.46	1.18	0.80
II	15	12.4	66.0	11.9	5.67	4.05

*To whom correspondence should be addressed at Department of Chemistry, The University of Auckland, PB 92019, Auckland, New Zealand.
E-mail: cj.oconnor@auckland.ac.nz



SCHEME 1

= 7, pyridine), suggesting a catalytic preference for hydrolysis at the *sn*-1 position, but there is no literature precedent for such catalysis by a pregastric enzyme.

The values obtained from Example II are conservatively small, especially for the value in $CHCl_3$, which was likely to have been affected by inability to remove pyridine completely from the original sample. Importantly, however, reversal of sign of specific rotation in the two solvents is confirmed, and the positive value in pyridine is twice that quoted (5). This reference includes an explicit caveat that the values of $[\alpha]_D$ were "submitted with reservations" as to the purity of the "d-α,β-dibutyrin" preparation and the extent of its racemization, but these reservations have subsequently been disregarded. The possibility that the sign of optical rotation might be dependent on solvent has been little documented in the literature since 1959, when Baer and Mahadevan (6) determined $[\alpha]_D$ for 1,2-didecanoyl-*sn*-glycerol in six different solvents. The value of $[\alpha]_D$ for α,β-dibutyrin in $CHCl_3$ (c = 9.77) was zero (5), suggesting that racemization had occurred. No other data for *sn*-1,2-dibutyrylglycerol are available, and their absence probably reflects the ease of acyl transfer in this short-chain species which makes production of enantiometrically pure material difficult.

The general pattern of asymmetries in milk fat distribution is common to all mammalian milk fats, but porcine and human milk fat contain no $C_{4:0}$ and $C_{6:0}$ ester linkages. Of the 8.0 and 13.8 mol% total fatty acids present in ovine and bovine milks, respectively, all short-chain acids are present at *sn*-3, while $C_{16:0}$ and $C_{18:0}$ acids are predominantly at *sn*-1 (7). We have shown that pregastric lipases preferentially liberate short-chain fatty acids from symmetric monoacid triacylglycerols and from milk lipids (8). These present data confirm that preferential release of short-chain fatty acids from asymmetric substrates is due to the combined effect of their chain-length and position.

Stereoselectivity at *sn*-3 has previously been observed only for lingual (pregastric) human and rat lipases (2). ^{13}C NMR spectroscopy identified compound **2** as a 1,2(2,3)-dibutyrylglycerol. The data for optical rotation have further identified **2** as the *sn*-1,2-species and made clear that lamb pregastric lipase shows *sn*-3 specificity for hydrolysis of a short-chain lipid.

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