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Short communication

Preparation and liquid chromatographic analysis of propanediol fatty acid esters

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Abstract

Procedures were developed for the synthesis, purification and analysis of propanediol (PD) esters of *n*-fatty acids (FA). Mono- (MAPD) and di-acylated (DAPD) species were synthesized from PD and FA using an immobilized lipase (*Candida antarctica* B) in *tert.*-butanol. MAPD and DAPD were isolated using silica gel column chromatography as ~95% pure preparations. Normal phase gradient LC provided for resolution of MAPD, DAPD and FA. $UV_{220\ nm}$ detection provided a detection limit of about 1 μg, and a linear response range of up to 2000 μg. Response factors were determined for MAPD, DAPD and FA components comprised of *n*-fatty acyl lengths of 4–16. © 1997 Elsevier Science B.V.

Keywords: Propanediol fatty acid esters; Fatty acids

1. Introduction

Fatty acyl esters of propanediols are important functional components as emulsifiers for the food industry [1,2] and in the manufacture of personal care products [3] and detergents [4]. Propanediol esters are used also as plastics additives [5] and in pharmaceutical industries and organic and polymer synthesis [6]. Although propanediol esters are synthesized industrially using chemical catalysts at elevated temperatures, recent advances in microaqueous enzymology [7–10] have identified lipase-mediated ester synthesis, transfer and exchange reactions as alternative approaches. Enzymic methods of synthesis present comparative advantages in terms of enhanced reaction selectivity (toward fatty acid and alcohol) and reduced by-product formation.

An obvious requirement for initiating studies on lipase-mediated synthesis of propanediol fatty acid esters is a method to afford analysis of reaction products that is suitable for fatty acid substrates encompassing an acyl carbon number range (4–18) representative of those commonly found in nature. Although there are reports of a GLC method for analysis of propylene glycol monoesters [11], GLC methods for separating components with free alcohol functional groups usually require prior derivatization into trimethylsilyl ether adducts [12]. Furthermore, a computer-assisted search of literature dating back to 1972 revealed only one citation where LC analysis of mixed acylglycerols could also be applied to the analysis of propanediol fatty acid esters [13]. However, no details of this LC method as applied to propanediol fatty acid esters were offered, and it is obvious that fatty acids and diacylated propane diols would co-elute using the procedures outlined in [13]. Thus, the objective of this work was to develop an LC method for direct quantitative analysis of pro-

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panediol fatty acid esters (PDFAE) in the presence of fatty acids. To achieve this objective, acylated propanediols were required as standards. Because these compounds are not commercially available, a prerequisite of this work was to synthesize and purify acylated propanediols, of varying degree of esterification with fatty acids, encompassing the range of acyl chain lengths typically found in natural fats and oils.

Table 1
Enzymic reaction yields of acylated propanediol species

Fatty acid substrate	mol% in product mixture		
	FA	MAPD	DAPD
Butanoic	29	55	16
Octanoic	21	62	18
Dodecanoic	29	60	11
Hexadecanoic	39	57	4.4

2. Experimental

2.1. Chemicals

All chemicals were obtained from either Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). An immobilized lipase (EC 3.1.1.3, triacylglycerol acylhydrolase; Novozym 435, *Candida antarctica* B) was a gift from Novo Nordisk Bioindustrials (Franklinton, NC, USA). All water used was deionized and glass-distilled. All solvents used were HPLC-grade.

2.2. Preparation of 1,3-propanediol fatty acid esters (PDFAE)

Our initial attempts to prepare mono- and di-fatty acid esters of 1,3-propanediol (PD) using fatty acid anhydrides in pyridine at 70°C or by refluxing at 180°C for 10 h did not result in sufficient yields of PDFAE, especially the diacylated derivative. As an alternative procedure, we prepared the esters enzymically, using 200 mg Novozym 435 with 0.5 *M* *n*-fatty acid (FA) and 0.75 *M* PD in 20 ml *tert.*-butanol with orbital shaking at 300 rpm and 15 h incubation at 50°C. Typical product yields obtained by this synthesis, as analyzed by the LC method described in this paper, are provided in Table 1.

2.3. Isolation and purification of PDFAE

To isolate mono- and di-acyl PD (MAPD and DAPD), the reaction mixture was first filtered to remove enzyme and then solvent (*tert.*-butanol) was removed using a rotary vacuum evaporator at 80°C. The residue was dissolved in a small volume of chloroform and this solution was washed at least thrice with an equal volume of water to remove residual *tert.*-butanol and PD. After removal of chloroform with a rotary vacuum evaporator at 60°C, the residue was dissolved in ca. 3 ml hexane–acetone–ether (8:1:1, v/v/v) and then applied to a silica gel (grade 9385, 230–400 mesh, 60 Å; Merck, Darmstaat, Germany) column. Components were eluted isocratically with this same solvent at 21–23°C under ca. 20 kPa air pressure, and composition of each fraction was qualitatively assessed using TLC on silica gel (250 μm; Whatman, Fairfield, NJ, USA) with hexane–acetone–ether (6:1:3, v/v/v) as developing solvent and visualization by iodine staining. R_F values for fatty acid (FA), MAPD and DAPD were 0.67–0.75, 0.41–0.50 and 0.84–0.88, respectively. Fractions that were apparently pure in either MAPD or DAPD were pooled and solvent was removed using a rotary evaporator at 80°C. Samples were finally dissolved in hexane–ethanol (2:1, v/v), and purity was assessed using the LC method described in Section 2.5.

2.4. Verification of purity of PDFAE

^{1}H NMR analysis was done using a Bruker model AM-300 (300 MHz) spectrometer. Chemical shifts (δ, ppm) are reported using trimethylsilane as internal standard ($\delta = 7.26$) in $CDCl_3$. ^{1}H NMR analysis of the purified MAPD fraction revealed the following chemical shifts, δ: 4.21 (*t*, $J = 6.6$ Hz, $-CH_2-O-$acyl, 2H), 3.66 (*t*, $J = 5.9$ Hz, $-CH_2-OH$, 2H), 2.28 (*t*, $J = 8.1$ Hz, $-OCO-CH_2-$aliphatic, 2H), 2.05 (*br*, $-CH_2-OH$, 1H), 1.84 (*quintet*, $J = 5.9$ Hz, $HOCH_2-CH_2-CH_2O-$acyl, 2H), 1.59 (*quintet*, $J = 7.3$ Hz, $-OCO-CH_2-CH_2-$aliphatic, 2H), 1.23 (*m*, $-OCO-$

CH_2–CH_2–$(CH_2)_8$–CH_3, 16H), 0.85 (*t*, J=6.7 Hz, –CH_3, 3H). ^{1}H NMR analysis of the purified DAPD fraction revealed the following chemical shifts, δ: 4.15 (*t*, J=6.1 Hz, –CH_2–O–acyl, 4H), 2.30 (*t*, J=7.6 Hz, –OCO–CH_2–aliphatic, 4H), 1.97 (*quintet*, J=6.3 Hz, CH_2–$(CH_2O$–acyl$)_2$, 2H), 1.62 (*quintet*, J=7.2 Hz, –OCO–CH_2–CH_2–aliphatic, 4H), 1.26 (*m*, –OCO–CH_2–CH_2–$(CH_2)_8$–CH_3, 32H), 0.88 (*t*, J=6.8 Hz, –CH_3, 6H).

2.5. LC method for quantifying FA, MAPD and DAPD

LC was done using a silica column (Econosil, 250×4.6 mm, 5 μm; Alltech Associates, Deerfield, IL, USA) and a solvent elution program (Fig. 1) delivered by Model 510 pumps with gradient control with peak integration achieved using Baseline 810 software (Waters Associates, Milford, CT, USA). Component detection was by $UV_{220\ nm}$ (Model 454, Waters), restricting solvent selection to UV-transparent solvents, such as hexane and *n*-propanol.

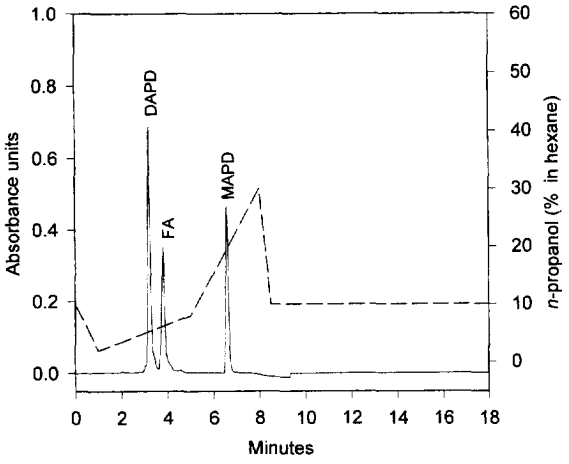

Fig. 1. High-performance LC analysis of propanediol fatty acid esters. Gradient elution program is indicated by dashed line (% *n*-propanol in hexane) and detector response is represented by solid line. Flow-rate was 1.0 ml min^{-1}, except between the 5.0 and 8.0 min interval where it was programmed linearly from 1.0 to 2.0 ml min^{-1}, and between the 8.0 and 8.5 min interval where it was programmed linearly from 2.0 to 1.0 ml min^{-1}. Sample injection volume was 20 μl (in hexane–ethanol, 2:1, v/v).

3. Results and discussion

3.1. Preparation of MAPD and DAPD standards

MAPD production was favored over DAPD by the enzymic procedure (Table 1). Although we used Novozym 435 to mediate synthesis of MAPD and DAPD standards, there are other lipases capable of this reaction [6,11,14]. Reaction yield was considered sufficient for preparing standards for subsequent LC studies.

3.2. Silica gel column chromatography

Typically, DAPD and FA eluted as pure (as indicated by TLC and iodine staining) components at 72–90 and 126–162 ml, respectively, for all fatty acid derivatives. For the MAPD species, the butanoyl derivative eluted at 324–342 ml and other (8, 12, 16 acyl carbon lengths) MAPD derivatives eluted at 198–234 ml. Total separation time was 3.5–5.0 h. Typical yields of purified MAPD and DAPD were 50–60 and 30–40%, respectively, of the amounts loaded onto the column. MAPD and DAPD obtained in this manner were about 95% pure as indicated by the LC method described in Section 2.5 and Fig. 1. The impurity in both of these fractions was FA. Determination of response factors for the contaminating FA allowed for correction and accurate quantitation of both PDFAE species by LC.

It was our experience that choice of silica gel was important to the success of the silica gel preparative chromatography step. Although we evaluated only two sources of silica gel, reproducible results were always obtained using the Merck silica gel product, whereas unsatisfactory reproducibility was obtained with the other.

3.3. Quantitation of FA, MAPD and DAPD by the LC method

A typical LC chromatogram shows the resolution of FA, MAPD and DAPD (Fig. 1). The initial minute of the reverse gradient was necessary to resolve FA and DAPD. Several other isocratic and alternative gradient programs during this initial period were not capable of resolving FA and DAPD, for reasons that remain unknown to the authors.

Retention times were affected by the acyl chain length of the fatty acid constituent with the expected pattern of adducts of longer acyl chain lengths having reduced retention times (Table 2). With this method, FA, MAPD and DAPD populations derived collectively from acyl chain lengths of 4–16 could be resolved, provided that column integrity is maintained to afford resolution of mixed DAPD and FA populations. We did not evaluate HPLC columns other than the one described, and found that over time, performance of the column used in the present context became compromised to the point that resolution of FA, MAPD and DAPD populations of mixed fatty acyl constituents was not possible. However, resolution of FA, MAPD and DAPD mixtures comprised by a single fatty acyl constituent was maintained for several months of relatively heavy use.

Relative response factors for FA, MAPD and DAPD constituents as affected by fatty acyl chain length indicated that generally, sensitivity was increased for more fully acylated derivatives and as fatty acyl chain length increased (Table 3). Detection limits were estimated (conservatively) to be of the order of 1 μg (others have reported 100 ng; [15]), and a linear response was obtained at levels up to about 2000 μg.

3.4. General discussion

We chose UV detection over alternative RI and mass detection methods for several reasons. Compared to RI detection, UV detection can accommodate solvent gradient programs, which was considered a priori to be essential to achieve the separation reported in this paper. Compared to mass detection systems, UV detection has the advantages in the present context in that it provides for a linear response over a broad range of analyte concentrations, is in more common laboratory use and can detect all target components of the present analysis. With regard to the last consideration, operation of a mass detector (Model ELSD IIA; Varex, Rockland, MD, USA) at 80°C using N_2 as the nebulizing gas at 50 ml min^{-1}, FA, MAPD and DAPD comprised of 4–12 acyl carbon residues were partially or wholly "transparent" to detection. The interdependence of detector (evaporator) temperature and analyte volatility presents problems in terms of varying detector response or sensitivity when analytes encompassing a broad range of boiling points are intended to be analyzed using mass detection [16].

We also chose to use a detection wavelength of 220 nm. While 200–210 nm is more sensitive for lipid analysis than 220 nm [17,18], the use of 220 nm is more specific for the carbonyl/carboxyl functional group with minimum interference from other functional groups, especially fatty acid unsaturation [15]. Under the conditions chosen for the present study, the limit of detection using $UV_{220\ nm}$ rivalled that reported for RI and mass detection [16,19].

The procedures of column chromatography (preparative) and LC (anaytical) developed here for

Table 2
Retention times for reaction products of fatty acid with propanediol

Fatty acyl constituent	Retention times (min)		
	FA	MAPD	DAPD
Butanoic	3.9	7.5	3.3
Octanoic	3.7	6.6	3.2
Dodecanoic	3.6	6.2	3.1
Hexadecanoic	3.4	5.9	3.0

Retention times were generally reproducible within ±0.1 min, using the LC program described in Fig. 1.

Table 3
Relative response factors of fatty acid and acylated propane diols

Fatty acyl constituent	Relative response factors[a]		
	FA	MAPD	DAPD
Butanoic	1.000 (1.000)[b]	0.754	0.459
Octanoic	1.000 (0.687)	0.964	0.471
Dodecanoic	1.000 (0.623)	0.943	0.275
Hexadecanoic	1.000 (0.599)	0.784	0.188

[a] Relative response factors, for UV detection at 220 nm, for adducts of each fatty acid constituent (each row) is expressed relative to the free acid.

[b] Relative response factors for each fatty acid (FA column) is expressed relative to butanoic acid. Coefficient of variation for determination of response factors was 5–10%.

PDFAE (and FA) were also suitable for preparation and analysis of mixtures of acylglycerols and FA.

4. Conclusion

Simple methods were developed for the production, isolation and LC analysis of fatty acyl esters of propanediols. Sensitivity and linear response ranges rivalled existing LC methods for analyzing functionally similar compounds (acylglycerols). These methods involve equipment and procedures in common laboratory practice.

Acknowledgments

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