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DIASTEREOMERIC SEPARATIONS OF NATURAL GLYCERO DERIVATIVES AS THEIR 1-(1-NAPHTHYL)ETHYL CARBAMATES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Diastereomeric separations of several natural glycero derivatives as their 1-(1-naphthyl)ethyl carbamates have been performed by high-performance liquid chromatography (HPLC). The HPLC diasteromeric separations of monoalkyl-, dialkyl- and diacyl-*sn*-glycerols are discussed. By this method it was also possible to prepare derivatives and perform enantiomeric analysis in the nanomole range. The expected structures of the various samples were established by mass spectrometric studies of HPLC eluates.

INTRODUCTION

There is a considerable interest in developing reliable and sensitive methods for the enantiomeric analysis of various glycerolipids[1]. In particular, interest has been concentrated on the analysis of various diacyl-*sn*-glycerols[2], because of their importance in biosynthesis and because they are degradation products from the lipolysis of triacylglycerols and phosphatides. In addition, dialkyl-*sn*-glycerols in various bacteria[3,4] and monoalkyl-*sn*-glycerols, which are widespread in most tissues of man and animals[5], have been studied in this respect.

For the enantiomeric analysis of various *sn*-glycero derivatives, optical methods such as optical rotatory dispersion and circular dichroism techniques have frequently been employed[6]. The main drawback of these methods is their lack of sensitivity and, consequently, the large amount of sample required. In most instances this has restricted the number of investigations of biochemical interest.

We have recently investigated the possibility of using capillary gas chromatography for the enantiomeric analysis of diastereoisomeric acylglycerols formed by

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esterification of 1,2-diacylglycerols with chiral small acids[7]. Several chiral reagents were used, including 2-phenylselenopropionic acid, α-methoxy-α-trifluoromethylphenylacetic acid (Moscher's MTPA reagent) and α-methoxy-α-methyl(pentafluorophenyl)acetic acid. The diethanoyl diastereoisomers with any chiral acid exhibited baseline separation on a 25-m fused-silica capillary column (coated with SE-54). However, the separastion decreased markedly with increasing chain length of the glycerol acyl units and, with the 1,2-didecanoylglycerol esters, only slight separation was achieved. The detection limits of the enantiomeric analyses were typically in the picomole or femtomole range employing flame ionization or selected ion monitoring detection, respectively.

These findings led us to focus our interest on high-performance liquid chromatography (HPLC) for enantiomeric separations. A procedure often used in the analysis of enantiomeric mixtures of various hydroxy-containing compounds is to convert them into the corresponding carbamates with an enantiomerically pure amine[8–11]. This paper describes the HPLC separation of enantiomeric diacyl-, O-dialkyl and O-monoalkyl-*sn*-glycerols as diastereomeric carbamate derivatives of 1-(1-naphthyl)ethylamine. Mass spectrometric studies of the various samples confirmed their proposed structures.

EXPERIMENTAL

Chemicals

All solvents were of the highest quality available, and were distilled prior to use. (*S*)-1-(1-Naphthyl)ethylamine was purchased from K and K Labs. (U.S.A.). The corresponding isocyanates were prepared by refluxing the amine with an excess of phosgene in benzene (12.5 wt.-%) for 3 h. The reaction products were distilled under reduced pressure, b.p. 106–108°C (0.15 mmHg). Racemic and enantiomeric 1,2-diacyl-, 1,2-O-dialkyl- and 3-O-alkylglycerols were prepared as described elsewhere[7].

Derivatization procedures

The diastereomeric carbamates were prepared via one of two procedures[8].

Procedure A. A mixture of racemic 3-O-hexadecylglycerol (100 mg, 0.316 mmole) and (*S*)-1-(naphthyl)ethyl isocyanate (125 mg, 0.633 mmole) in 7 ml of a solution of N,N-dimethylethanolamine in dry toluene (1 wt.-%) was heated at 80°C for 36 h. After cooling to room temperature, the reaction mixture was evaporated under a stream of nitrogen and dissolved in the appropriate mobile phase for HPLC.

Procedure B. A solution of *rac*-1,2-dilauroylglycerol (50 mg, 0.110 mmole) in 500 μl of dry toluene and 175 μl of triethylamine was added dropwise to an ice-cold stirred solution of 200 μl of phosgene (12.5 wt.-% in benzene) in 500 μl of dry toluene. After the addition was completed, stirring was continued for 1 h at 0°C and then for 3 h at room temperature. The reaction mixture was then concentrated under reduced pressure (*ca.* 50 mmHg) with heating (40–50°C).

To the crude chloroformate, (*S*)-1-(1-naphthyl)ethyl amine (19 mg, 0.110 mmole) in 100 μl of dry toluene and 175 μl of triethylamine was added dropwise with stirring. After the addition was completed, stirring was continued overnight at room temperature.

The reaction mixture was diluted with 5 ml of diethyl ether and washed with

water, 2.5 *M* hydrochloric acid, water, saturated sodium hydrogen carbonate solution and finally water. After drying the organic layer over anhydrous sodium sulphate, followed by filtration, the solvent was removed by evaporation. The mixture of carbamates was dissolved in the appropriate mobile phase for HPLC.

In a different experiment the preparation was scaled down to the nanomole level. A 10 nmole amount of 1,2-O-ditetradecylglycerol was then reacted as described for the preparation of chloroformate in procedure B, with the exception that 5-fold excesses of reagents were used. The reaction mixture was left overnight and treated as described.

High-performance liquid chromatography

The separations were performed with an LDC Consta Metric III HPLC system equipped with an LDC monitor operating at 254 nm or a Shimadzu SPP-2AC monitor operating at 280 nm. The samples were applied to the chromatograph by a Rheodyne 7125 sample loop injector with a volume of 20 μl for the analytical samples and 100 μl for the preparative work.

A 50 cm × 10 mm I.D. commercial 5-μm microparticle silica gel column (Scandinavia GeneTec, Sweden) was used at a flow-rate of 3.6 ml/min. The mobile phase given under Results and discussion was modified with 1% of 2-propanol.

Mass spectrometry

The mass spectrometer was a Finnigan 4021 quadrupole instrument with a mass spectral data acquisition system, operating at an electron energy of 70 eV. The ion source temperature was 250°C (electron impact and chemical ionization). Ammonia was used as the reagent gas (purity 99.95%) and the pressure was 0.5 Torr.

The samples were introduced into the ion source with a heated direct inlet probe.

RESULTS AND DISCUSSION

Preparation of derivatives

The separation of diastereomeric carbamates on silica and alumina columns, and the underlying mechanisms, have been previously reported[8]. In this work, the influence of the stereo structures of the substrate and reagent on the separation is discussed.

The dicarbamate derivatives of monoalkyl-*sn*-glycerols were prepared according to procedure A because of the two hydroxy groups present in the substrate. However, we found that in some experiments this method led to the formation of by-products, and sometimes required pre-separation prior to analysis. This has also been observed by others[12].

For the preparation of dialkyl-*sn*-glycerol carbamate derivatives, either method can in principle be used; we need exclusively procedure B. Analysis could also conveniently be carried out at the nanomole level.

For the preparation of diacyl-*sn*-glycerol carbamates, procedure B was exclusively used, because the risk of possible acyl migration is reduced to the minimum. Recently, it has been reported[13] that procedure A, including a 200-fold excess of the reagent, can also be used with no acyl migration at the nanomole level.

TABLE I

HPLC DATA FOR DIASTEREOMERIC SEPARATIONS OF THE ALKYL-*sn*-GLYCEROLS INVESTIGATED AS THEIR (*S*)-1-(1-NAPHTHYL)ETHYL CARBAMATES

Mobile phase, *n*-hexane–ethyl acetate; silica gel (5 μm) column, 50 cm × 10 mm I.D.; t = adjusted retention times (t_0 = 7.5 min).

Derivative	*Configuration*	*Proportions of mobile phase components*	t_1	t_2	α	R_s
1,2-O-Ditetradecyl	*rac*	85:15	5.00	5.50	1.11	1.37
	rac	90:10	12.00	13.45	1.12	2.26
	rac	95:5	20.90	28.15	1.35	3.15
1,2-O-Dihexadecyl	*rac*	85:15	4.35	4.90	1.14	1.53
		90:10	12.10	13.45	1.11	2.35
	sn-1,2	85:15	–	4.90		
3-O-Tetradecyl	*rac*	85:15	3.60	4.35	1.21	2.50
	rac	90:10	4.20	5.30	1.26	3.67
	sn-3	85:15	3.60	–		
3-O-Hexadecyl	*rac*	85:15	5.30	5.80	1.09	1.67
	rac	90:10	9.70	10.75	1.10	2.33

Diastereomeric separations of 1,2-O-dialkyl- and 3-O-alkyl-sn-glycerols

The results for the O-alkylglycerols investigated are summarized in Table I.

Racemic 1,2-O-ditetradecyl- and 1,2-O-dihexadecylglycerol as (*S*)-1-(1-naphthyl)ethyl carbamates exhibited excellent separation of the two diastereomeric peaks, with a separation factor (α) of 1.14 and a resolution (R_s) value of 1.53 on a silica column with *n*-hexane–ethyl acetate (85:15, v/v) as the mobile phase (Fig. 1).

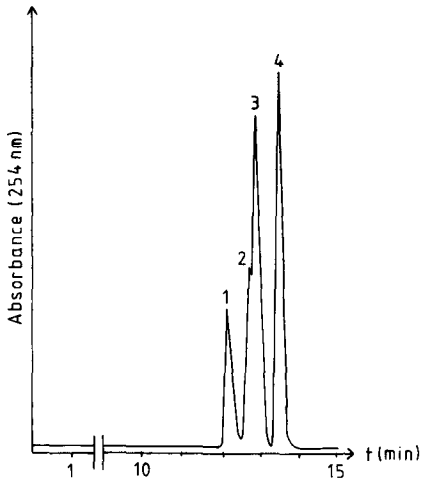

Fig. 1. Chromatograms of diastereomeric (*S*)-1-(1-naphthyl)ethyl carbamates of (1) 2,3-O-hexadecyl-, (2) 1,2-O-hexadecyl-, (3) 2,3-O-ditetradecyl- and (4) 1,2-O-ditetradecyl-*sn*-glycerol. Mobile phase: *n*-hexane–ethyl acetate (85:15, v/v).

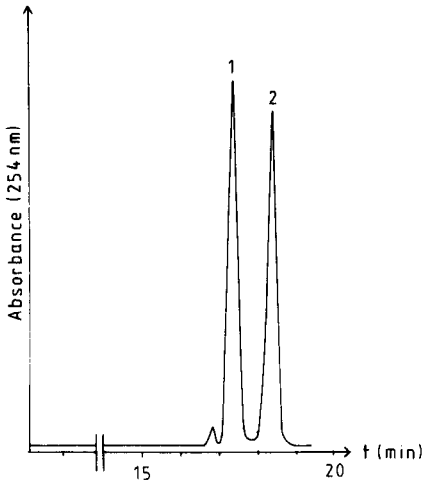

Fig. 2. Chromatograms of diastereomeric (*S*)-1-(1-naphthyl)ethyl carbamates of (1) 3-O-hexadecyl- and (2) 1-O-hexadecyl-*sn*-glycerol. Mobile phase: *n*-hexane–ethyl acetate (85:15, v/v).

Changing the proportions of the solvents in the mobile phase to 90:10 (v/v) increased R_s to 2.35 with an almost constant α value. A further increase in the *n*-hexane content gave a R_s value of 3.15 for the ditetradecyl isomer, but the retention times increased to unacceptable levels (*cf.*, Table I).

By choosing the 85:15 (v/v) composition of the mobile phase, various 1,2-O-dialkylglycerols could also be separated owing to differences in the chain length of the alkyl moiety (Fig. 1).

Our results indicate that an increase in the size of the alkyl moiety decreased the retention times (Table I).

HPLC of the dicarbamate derivatives of 3-O-tetradecyl- and 3-O-hexadecyl-glycerols resulted in well resolved diastereomeric peaks (Fig. 2), with approximately the same α and R_s values in the same two mobile phases as used for the dialkyl analogues. Also with these derivatives, it was possible to achieve a separation according to the chain length of the alkyl moiety. In contrast to the results obtained above, the results indicate that for these compounds an increase in the size of the alkyl moiety also increased the retention times (Table I).

The steric configurations of the eluted derivatives were established by comparison with enantiomerically pure glycerol carbamate derivatives of (*S*)-1-(1-naphthyl)ethylamine (Table II).

TABLE II

ABSOLUTE CONFIGURATIONS OF THE ELUTED DIASTEREOMERIC CARBAMATES OF 1,2-O-DIALKYL- AND 3-O-MONOALKYL-*sn*-GLYCEROL DERIVATIVES OF (*S*)-1-(1-NAPHTHYL)ETHYLAMINE

First analyte	*Second analyte*
2,3-O-Dialkyl-*sn*-glycerol	1,2-O-Dialkyl-*sn*-glycerol
3-O-Monoalkyl-*sn*-glycerol	1-O-Monoalkyl-*sn*-glycerol

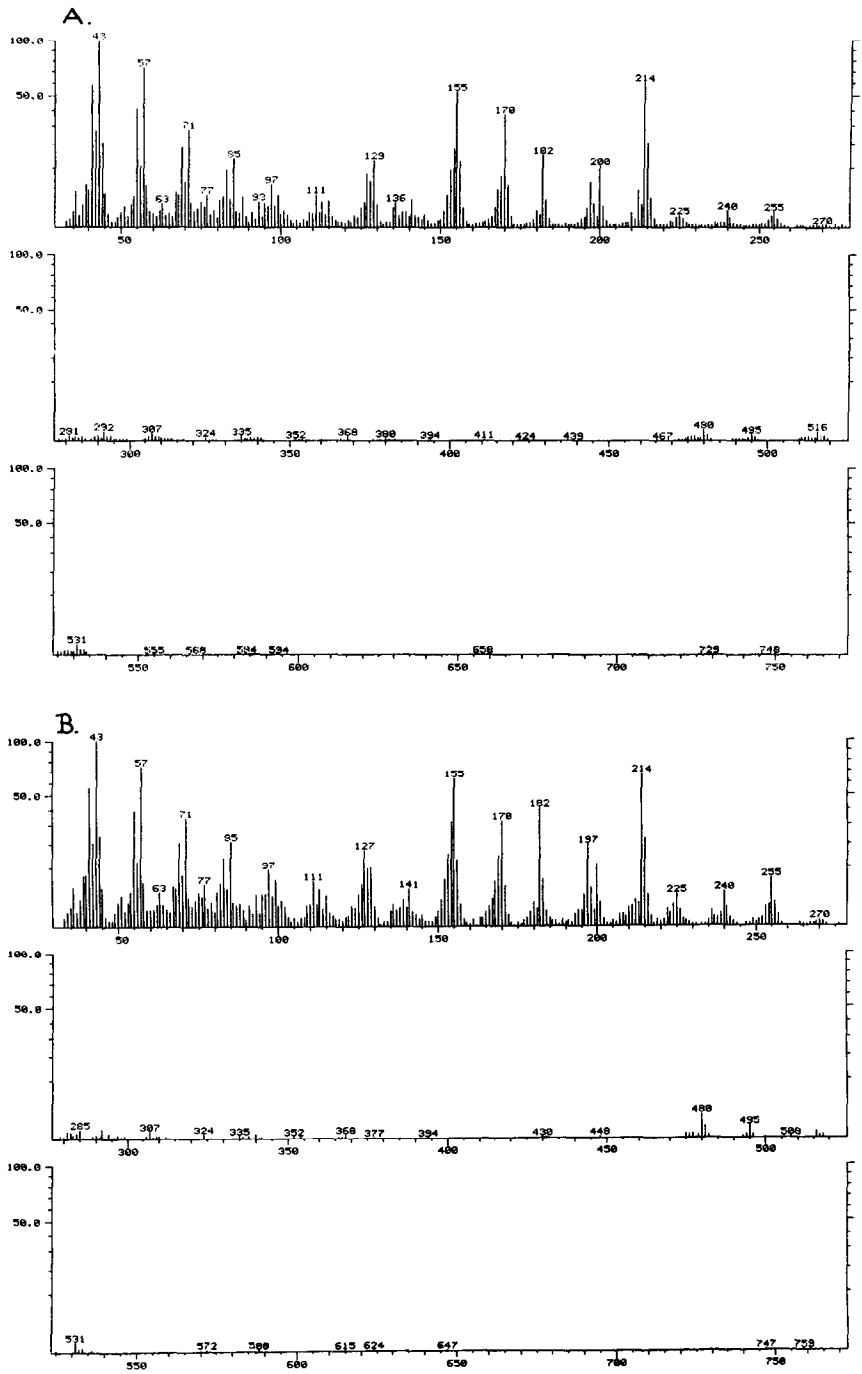

Fig. 3. Mass spectra (EI) of (A) first analyte and (B) second analyte of the (*S*)-1-(1-naphthyl)ethyl dicarbamate of 3-O-hexadecylglycerol.

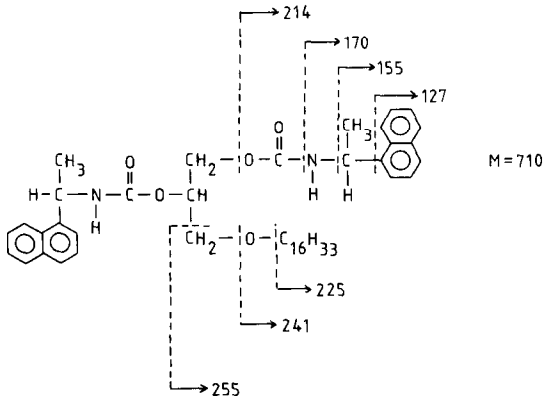

Fig. 4. Summary of fragmentation of (*S*)-1-(1-naphthyl)ethyldicarbamate of 3-O-hexadecyl-*sn*-glycerol.

The expected structures of the various samples were established by mass spectrometric studies of the HPLC eluates. Mass spectra were recorded using electron impact ionization (EI) and chemical ionization with ammonia as the reactant gas (CI-NH_3). The EI spectra of the investigated isomeric O-alkylglycerol derivatives exhibited no molecular ion (Fig. 3). The spectrum is dominated by the 1-(1-naphthyl)-ethyl carbamate fragmentation and of the alkyl moiety (Fig. 4). The CI spectra, on the other hand, showed molecular-related ions at *m/z* 682 (M) and *m/z* 700 (M + 18) for the 1,2-O-ditetradecylglycerol derivatives, whereas corresponding ions were absent for the monoalkyl isomers. The most dominant ions in CI, for both isomers, are those at *m/z* 155 (= 100%) and *m/z* 172 (= 95%).

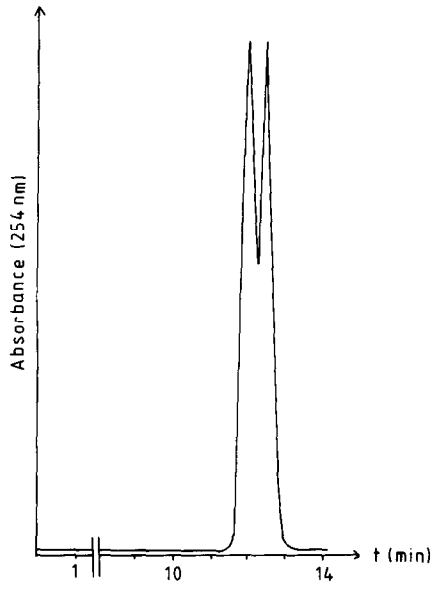

Fig. 5. Chromatogram of diastereomeric (*S*)-1-(1-naphthyl)ethyl carbamate of *rac*-1,2-dilauroylglycerol. Mobile phase: *n*-hexane–ethyl acetate (85:15, v/v).

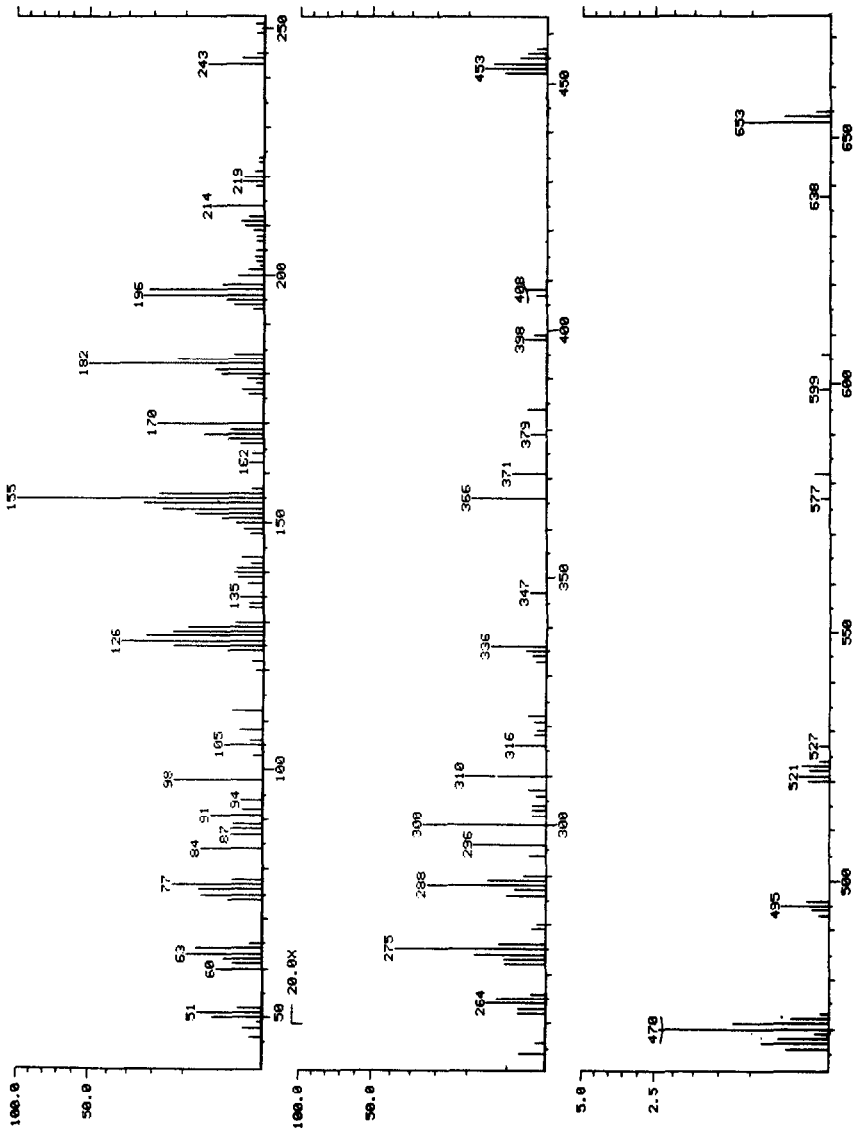

Fig. 6. Mass spectrum (EI) of the 1-(1-naphthyl)ethyl carbamate of 1,2-dilauroylglycerol. $(M)^+ = 653$.

Diastereomeric HPLC separation of 1,2-diacyl-sn-glycerol

The HPLC separation with *n*-hexane–ethyl acetate (85:15, v/v) of the 1-(1-naphthyl)ethyl carbamate derivatives of 1,2-dilauroyl-, 1,2-dimyristoyl-, 1,2-dipalmitoyl- and 1,2-distearoylglycerol produced chromatograms with two major peaks, in a 1:1 ratio, with and α value of 1.10 and R_s = 0.75 (Fig. 5). Clearly baseline separation was not obtained, in contrast to the 1,2-O-dialkylglycerol derivatives with the same α value. We are not able to explain these discrepancies.

The EI and CI-NH_3 mass spectra of the eluates were in agreement with the proposed structures and were, as expected, pairwise identical. In contrast to the spectra obtained for the alkylglycerol derivatives, a small molecular ion was obtained for the diacyl derivatives in EI (Fig. 6). Characteristic fragmentations of the 1,2-diacylglycerol moiety, identical with those of analogous acylglycerols described by others[14], were observed.

The separation efficiency was shown to be extremely solvent dependent and, in addition, required activation of the silica gel column with acetone to obtain the optimal conditions. Small changes in the mobile phase composition, *e.g.*, the addition of a modifier such as 2-propanol, caused inferior diastereomeric separations. In the absence of a modifier, reproducible retention times were difficult to achieve, and for this reason it was not possible to establish the absolute configuration of the eluates with optically pure samples.

The retention times decreased with increasing chain length using *n*-hexane–ethyl acetate as the mobile phase. Our results, together with the finding of Pirkle and Hauske[8], indicate that 1,2-diacylglycerol derivatives can be fully resolved with respect to chain length by changing to other column materials and solvents. Work to improve the diastereomeric separation is in progress.

For all types of glycerides investigated, UV detection at 254 nm of the derivatives permitted determinations at the 1.5 nmole level A shift in wavelength to 280 nm approximately doubled the sensitivity. Fluorescence detection[11] should allow even higher sensitivity.

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