A Mild Approach to the Synthesis of *sn*-Glycerol 1,2-Di-γ-linolenate 3-Palmitate

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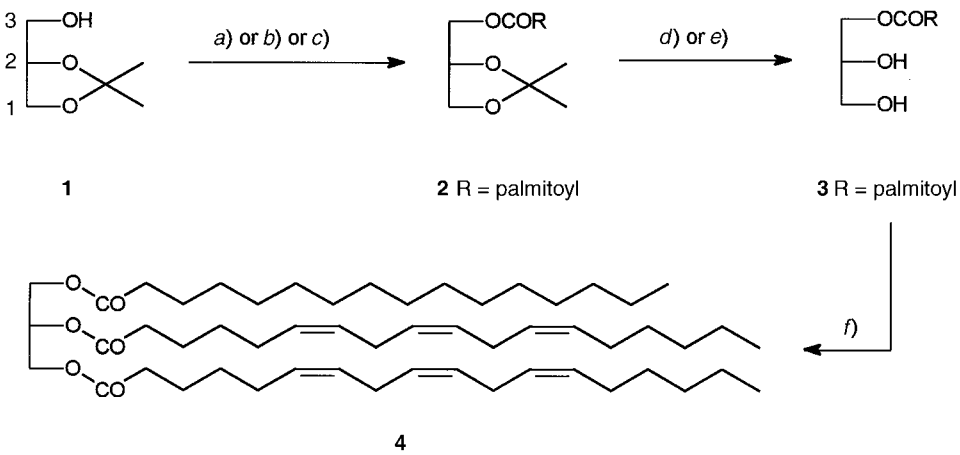
A synthetic approach comprising several studied modifications was applied to the preparation of *sn*-glycerol 1,2-di-γ-linolenate 3-palmitate (**4**). Thereby, a convenient and mild synthetic method was elaborated, affording **4** from 1,2-*O*-isopropylidene-*sn*-glycerol (**1**) in an average yield of 65–75% and analytically acceptable purity.

Introduction. – As part of a complex project focused on the investigation of fatty acids occurring in blackcurrant (*Ribes nigrum*) oil, attention has also been paid to a synthesis of triacylglycerols derived from polyunsaturated fatty acids. Among a number of such fatty acids, increasing evidence for a positive effect of γ-linolenic acid (=(6*Z*,9*Z*,12*Z*)-octadeca-6,9,12-trienoic acid; GLA) on clinical improvement in a number of serious diseases has been reported [1–5]. GLA represents an important component of the blackcurrant oil [6]. However, the commercial availability of triacylglycerols derived from GLA is still limited. Thus, a mild synthesis of *sn*-glycerol 1,2-di-γ-linolenate 3-palmitate (**4**), a not commercially available representative of the series of potential GLA-based glycerol derivatives, was elaborated. Compound **4** was requested as reference compound for the analysis of blackcurrant oil and of the products of its enzymic transformation. A synthetic approach published earlier by *Redden et al.* [7], which afforded **4** in high purity, was taken as the basis for the design of our procedure. It has been shown that almost always acyl migration from the *sn*-2 position to either of the terminal positions (*sn*-1 or *sn*-3) occurred, the opposite migration from the terminal positions (*sn*-1 and *sn*-3) to the *sn*-2 position taking place only to a limited extent [7]. In several earlier reports [8–10], the 1,2-acyl migration has been described under synthetic conditions that were not sufficiently mild. Therefore, the recent findings of *Redden et al.* [7] provided key information for our own strategy towards the synthesis of **4**.

Results and Discussion. – The general strategy towards the synthesis of **4** (see *Scheme*) requires as the first step the selective substitution of the free OH group of the 1,2-*O*-isopropylidene-protected *sn*-glycerol **1** by the palmitoyl functionality to yield **2**. The ensuing deprotection of the blocked OH groups of **2** should then result in the liberation of *sn*-glycerol 3-palmitate (**3**), which can be acylated with GLA to the target *sn*-glycerol 1,2-di-γ-linolenate 3-palmitate (**4**). Although this strategy looks very simple, several synthetic difficulties were encountered.

Scheme



a) RCOCl, benzene, DMAP; CC (silica gel). *b*) RCOCl, benzene, pyridine; CC (silica gel). *c*) RCOOH, DCC, DMAP, CCl_4; CC (silica gel). *d*) CF_3COOH, CH_2Cl_2, 0°, Ar; CC (silica gel). *e*) H_3BO_3, $CH_3OCH_2CH_2OH$, reflux; CC (silica gel). *f*) GLA, DCC, DMAP, CCl_4, 20°, Ar; CC (silica gel).

Three approaches [7][11][12] to the acylation of 1,2-*O*-isopropylidene-*sn*-glycerol (**1**) by palmitic acid (= hexadecanoic acid) or by its chloride were tested. Acylation of **1** by palmitoyl chloride in benzene in the presence of *N*,*N*-dimethylpyridin-4-amine (DMAP) according to *Jacobs et al.* [11] gave **2** in 72% yield, whereas acylation by palmitoyl chloride in benzene/pyridine according to *Buchnea* [12] afforded **2** in 88% yield, but in unsatisfactory purity. Best results were obtained with the method of *Redden et al.* [7]. Accordingly, acylation of **1** with palmitic acid was performed under mild conditions in the presence of dicyclohexylcarbodiimide (DCC) and DMAP as catalysts, furnishing **2** in 92% yield and in high purity. Another advantage of this method [7] was that palmitic acid itself could be used instead of the sensitive palmitoyl chloride.

A major difficulty arose in the second step of the planned synthesis. Indeed, removal of the protective group from **2** with CF_3COOH in CH_2Cl_2 at 0° under Ar [11] resulted in compound **3**, which was not pure enough even after column chromatography (silica gel), the impurity being most likely a 1,2-acyl-migration product. However, boric acid, a much weaker acid than CF_3COOH, in refluxing 2-methoxyethanol [7] was successfully used in this deprotection step affording **3** in sufficient purity. Finally, acylation of **3** was performed analogously to the previous acylation step [7] (GLA in the presence of DCC and DMAP in CCl_4 under Ar) and afforded the target **4** in 65–75% yield in high purity. An alternative acylation of **3** with the chloride of GLA was of limited use due to the low stability of the parent GLA.

The purity of compound **4** was checked by HPLC. As known analytical procedures [13][14] gave only unsatisfactory results when applied to **4**, a new method of HPLC analysis was used, involving, among other approaches, electron light scattering (ELS) detection of **4**; indeed, **4** displays no characteristic peak in the UV spectrum. Thus, reversed-phase HPLC with a chiral *Nucleodex β-OH* column (200 × 4 mm) and MeOH as eluent (*cf. Exper. Part*) revealed a single peak of **4** (t_R 5.7 min). This finding may be

caused either by an insufficient effectiveness of the chiral column or by the composition of the mobile phase (100% MeOH). Addition of H_2O to the mobile phase (5–20% H_2O/MeOH (*v*/*v*)) gave rise to an inconvenient increase of t_R, but no peak splitting was observed; the peak shape of **4** indicated that addition of H_2O to the mobile phase results in a reduced chance to detect chiral resolution of **4**. For the analytical requirements during the analysis of the blackcurrant-oil components, the reversed-phase HPLC analysis was, however, less satisfactory than an alternative HPLC on silica gel. Thus, HPLC of **4** with a *Biosphere Si 100* (silica-gel-filled) column (250 × 4 mm; 5 μm) in combination with a special gradient program (*cf. Exper. Part*), revealed again a single peak (t_R 16.1 min). This special gradient program was used for a series of HPLC analyses of different fractions (triacylglycerols, diacylglycerols, monoacylglycerols, and free fatty acids) obtained from blackcurrant oil and those obtained after its enzymic transformations and subsequent separation by column chromatography. The observed t_R of **4** (16.1 min) is in accordance with the t_R region of triacylglycerol derivatives found generally under the same HPLC conditions. The special gradient program used (*cf. Exper. Part*) is unusual; however, it is the result of a complex study, in which we focused on the separation of mixtures consisting of tri-, di-, and monoacylglycerols and free fatty acids which had to be performed within no more than 30–40 min. The special gradient program met exactly the conditions required for that study and resulted in a much better separation of mixtures than that obtained by the mobile phases referred to in [13] and [14].

Conclusion. – The general synthetic approach for the preparation of triacylglycerols derived from GLA was undertaken as part of the currently running project dealing with plant triacylglycerols of natural origin. The comparison of different methods for the preparation of triacylglycerols derived from polyunsaturated fatty acids clearly shows the advantages of the herein-described method, which is based on the work of *Redden et al.* [7]. The experience obtained during the synthesis of triacylglycerol **4** is useful for the handling and storing of other potentially labile triacylglycerols derived from GLA. Moreover, **4** represents an important reference compound for the HPLC analyses of triacylglycerols.

Experimental Part

General. Column chromatography (CC): silica gel (*Herrmann*, Köln-Ehrenfeld, FRG). TLC: Precoated silica-gel plates. HPLC: System built by *Watrex*, Czech Republic, and consisting of a *Thermoseparation-Products* instrument (*TSP*, USA) operated by a *Pentium* PC with an OS-2 WARP/PC-1000 software (*TSP*, USA), a *ConstaMetric 4100-Bio* pump (*TSP*, USA), a *SpectroMonitor 5000* UV DAD (*TSP*, USA), and an ELSD (*Polymer Laboratories*, USA); ELSD conditions: N_2 flow 0.8 ml · min^{-1}, evaporation temp. 70°, nebulizer temp. 40°. Reversed-phase HPLC: *Nucleodex β-OH* chiral column (*Macherey-Nagel*, FRG; 200 × 4 (i.d.) mm) with MeOH as a mobile phase, flow rate 0.3 ml · min^{-1}; silica-gel HPLC: *Biosphere-Si-100* column (*Watrex*, Czech Republic; 250 × 4 (i.d.) mm, particle size 5 μm) with a gradient program (40 min) consisting of solvents *A* and *B* at a flow rate of 0.4 ml · min^{-1}; solvent *A* = Et_2O/light petroleum ether/^{i}PrOH 48.15 : 48.15 : 3.7 (*v*/*v*), solvent *B* = light petroleum ether; gradient program: *a*) *A* (2%) + *B* (98%) for 2 min (isocratic), *b*) linear gradient change of the preceding eluent to *A* (27%) + *B* (73%) within 5 min, *c*) 6 min isocratic, *d*) linear gradient change of the preceding eluent to *A* (100%) + *B* (0%) within 7 min, *e*) 10 min isocratic, *f*) linear return to the starting eluent *A* (2%) + *B* (98%) within 10 min. IR Spectra: *Bruker IFS-88* instrument; $CHCl_3$ solns.; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Varian Unity-500* spectrometer (FT mode) at 499.8 (^{1}H) or 125.7 MHz (^{13}C); $CDCl_3$ solns. with Me_4Si as internal reference ($\delta = 0.0$ (^{1}H) or 77.0 (^{13}C; central line of the solvent signal)), *J* in Hz. Mass spectra: *VG* analytical instrument *ZAB-EQ* in a BEQQ configuration.

*1,2-*O*-Isopropylidene-*sn*-glycerol 3-Palmitate* (**2**). *Method A:* A soln. of **1** (1.063 g, 1 ml, 8.0 mmol), palmitoyl chloride (2.21 g, 2.4 ml, 8.0 mmol), and DMAP (1.0 g, 8.3 mmol) in dry benzene (103 ml) was stirred at r.t. and under Ar for 2 h. The org. soln. was then washed with 5% (*w*/*v*) $NaHCO_3$ soln. (2 × 10 ml), 0.1N HCl (2 × 10 ml), and brine (2 × 10 ml), dried ($MgSO_4$), and evaporated: 2.38 g of a crude product, which was purified by CC (petroleum ether/AcOEt 50:1 → 20:1): 1.555 g (71.5%) of pure **2**.

Method B: Palmitoyl chloride (4.14 g, 4.5 ml, 15.1 mmol) was added to a soln. of **1** (2.126 g, 2.0 ml, 16.1 mmol) in dry benzene (15.6 ml) and in dry pyridine (1.6 ml). The mixture was stirred at r.t. under Ar for 24 h, then diluted with Et_2O (15 ml), acidified with ice-cold 1N H_2SO_4 to remove pyridine, and washed with sat. $NaHCO_3$ soln. and H_2O. After drying (Na_2SO_4), evaporation afforded 6 g of a crude product. CC Purification gave 5.25 g (88.1%) of pure **2**.

Method C: Palmitic acid (6.15 g, 24.0 mmol) and DMAP (2.656 g, 21.8 mmol) were added to a soln. of **1** (2.877 g, 2.7 ml, 21.8 mmol) in CCl_4 (32.4 ml). A soln. of DCC (4.96 g; 24.0 mmol) in CCl_4 (32.4 ml) was added dropwise with stirring under Ar over 30 min (TLC monitoring). Precipitated dicyclohexylurea was filtered off and washed with CCl_4. The resulting soln. was concentrated and the crude residue purified by CC, affording 7.42 g (92.0%) of **2**, identical (by spectra) to **2** obtained according to *Methods A* and *B*. IR: 2989*w*, 2927*s*, 2872*s*, 1743*s*, 1467*w*, 1457*w*, 1381*w*, 1371*w*, 1213*w*, 1160*m*, 1083*w*. ^{1}H-NMR: 0.88 (*t*, *J* = 6.9, 3 H); 1.07 (*q*, *J* = 0.7, 3 H); 1.20–1.36 (*m*, 24 H); 1.43 (*q*, *J* = 0.7, 3 H); 1.62 (*m*, 2 H); 2.34 (*dd*, *J* = 7.3, 7.9, 2 H); 3.74 (*dd*, *J* = 6.1, 8.4, 1 H); 4.08 (*dd*, *J* = 6.5, 8.4, 1 H); 4.09 (*dd*, *J* = 5.9, 11.6, 1 H); 4.16 (*dd*, *J* = 4.7, 11.6, 1 H); 4.31 (*dddd*, *J* = 4.7, 5.9, 6.1, 8.5, 1 H). ^{13}C-NMR: 14.07 (*q*); 22.66 (*t*); 24.88 (*t*); 25.38 (*q*); 26.66 (*q*); 29.10 (*t*); 29.22 (*t*); 29.33 (*t*); 29.43 (*t*); 29.57 (*t*); 29.61 (*t*); 29.62 (*t*); 29.64 (*t*); 29.66 (*t*); 29.67 (*t*); 31.90 (*t*); 34.10 (*t*); 64.49 (*t*); 66.36 (*t*); 73.66 (*d*); 109.78 (*s*); 173.58 (*s*). EI-MS (pos.): 369 (5, $[M-H]^+$), 313 (100), 239 (5).

sn-Glycerol 3-Palmitate (**3**). *Method D:* A soln. of **2** (1.0 g, 2.7 mmol) in CH_2Cl_2 (42 ml) was stirred in the presence of CF_3COOH (2 ml) at 0° under Ar for 12 h. The resulting soln. was evaporated, and the traces of CF_3COOH were removed under high vacuum. The crude residue was purified by CC (silica gel; AcOEt/petroleum ether 3:7): 0.778 g (87.2%) of **3**.

Method E: Compound **2** (6.11 g, 16.5 mmol) and boric acid (1.03 g, 16.5 mmol) were added to 2-methoxyethanol (6.0 ml). The mixture was refluxed under a drying tube ($CaCl_2$) for 2 h (TLC monitoring). The mixture was cooled, Et_2O added (10 ml), the soln. washed well with distilled H_2O, dried ($MgSO_4$), and evaporated, and the crude residue purified by CC: 5.04 g (92.5%) of **3**, identical (by spectra) to **3** obtained by *Method D*. IR: 3619*w*, 3490*w*, 3021*w*, 2927*s*, 2855*s*, 1734*m*, 1466*m*, 1222*s*, 1174*w*, 1114*w*, 1050*w*. ^{1}H-NMR: 0.88 (*t*, *J* = 6.8, 3 H); 1.23–1.34 (*m*, 24 H); 1.63 (*m*, 2 H); 2.35 (*t*, *J* = 7.6, 2 H); 3.60 (*dd*, *J* = 5.9, 11.5, 1 H); 3.70 (*dd*, *J* = 4.0, 11.5, 1 H); 3.93 (*ddt*, *J* = 4.0, 4.6, 6.0, 6.0, 1 H); 4.15 (*dd*, *J* = 6.1, 11.6, 1 H); 4.20 (*dd*, *J* = 4.6, 11.6, 1 H). ^{13}C-NMR: 14.09 (*q*); 22.67 (*t*); 24.91 (*t*); 29.11 (*t*); 29.23 (*t*); 29.34 (*t*); 29.43 (*t*); 29.56 (*t*); 29.62 (*t*); 29.64 (*t*); 29.65 (*t*); 29.66 (*t*); 29.68 (*t*); 31.91 (*t*); 34.15 (*t*); 63.34 (*t*); 65.16 (*t*); 70.28 (*d*); 174.34 (*s*). FAB-MS: 331 (84, $[M+H]^+$), 313 (26), 239 (50), 225 (36).

sn-Glycerol 1,2-Di-γ-linolenate 3-Palmitate (**4**). GLA (37.5 mg, 0.135 mmol) and DMAP (14.8 mg, 0.123 mmol) were added to a soln. of **3** (20.0 mg, 0.0605 mmol) in CCl_4 (10 ml). Then a soln. of DCC (27.8 mg, 0.135 mmol) in CCl_4 (5 ml) was added dropwise within 30 min with stirring and under Ar (TLC monitoring). Precipitated dicyclohexylurea was filtered off and washed well with CCl_4. The filtrate was evaporated and the crude product purified by CC: 80.9 mg (70.4%) of **4**. IR: 3013*m*, 2956*m*, 2927*s*, 2872*m*, 1746*s*, 1216*m*, 1165*m*, 1100*w*. EI-MS (pos.): 595 (32, $[M-255]^+$), 573 (100), 313 (46). ^{1}H-NMR: 0.88 (*t*, *J* = 6.9, 6 H); 0.89 (*t*, *J* = 6.9, 3 H); 1.24–1.42 (*m*, 30 H); 1.67 (*m*, 4 H); 2.08 (*m*, 8 H); 2.32 (*m*, 6 H); 2.80 (*m*, 8 H); 4.14 (*dd*, *J* = 5.9, 11.9, 2 H); 4.30 (*dd*, *J* = 4.3, 11.9, 2 H); 5.26 (*tt*, *J* = 4.3, 4.3, 5.9, 5.9, 1 H); 5.30–5.44 (*m*, 12 H). ^{13}C-NMR 14.06 (*q*); 14.11 (*q*); 62.07 (*t*); 62.13 (*t*); 68.94 (*d*); 127.55 (*d*); 127.57 (*d*); 128.00 (*d*); 128.03 (*d*); 128.32 (*d*); 128.33 (*d*); 128.45 (*d*); 128.46 (*d*); 129.47 (*d*); 130.46 (*d*); 172.65 (*s*); 173.06 (*s*); 173.27 (*s*).

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REFERENCES

[1] D. F. Horrobin, *Prog. Lipid Res.* **1992**, *31*, 163.
[2] D. Wu, S. N. Meydani, in 'γ-Linolenic Acid, Metabolism and Its Roles in Nutrition and Medicine', Eds. Y. S. Huang, D. E. Mills, AOCS Press, Champaign, IL, 1996, Chapt. 9, p. 106.

[3] J. Eritsland, in 'Essential Fatty Acids and Eicosanoids', Eds. R. A. Riemersma, R. Armstrong, R. W. Kelly, R. Wilson, AOCS Press, Champaign, IL, 1998, p. 243.
[4] A. Nordoy, K. H. Bonaa, H. Nilsen, R. K. Berge, J.-B. Hansen, O.-C. Ingebretsen, in 'Essential Fatty Acids and Eicosanoids', Eds. R. A. Riemersma, R. Armstrong, R. W. Kelly, R. Wilson, AOCS Press, Champaign, IL, 1998, p. 256.
[5] W. Jiang, S. Hiscox, R. P. Bryce, D. F. Horrobin, R. E. Mansel, in 'Essential Fatty Acids and Eicosanoids', Eds. R. A. Riemersma, R. Armstrong, R. W. Kelly, R. Wilson, AOCS Press, Champaign, IL, 1998, p. 303.
[6] M. Vacek, M. Zarevúcka, Z. Wimmer, K. Stránský, B. Koutek, M. Macková, K. Demnerová, *Enzyme Microb. Technol.*, in press.
[7] P. R. Redden, X. Lin, D. F. Horrobin, *Chem. Phys. Lipids* **1996**, *79*, 9.
[8] R. G. Jensen, R. E. Pitas, *Adv. Lipid Res.* **1976**, 213.
[9] D. R. Kodali, *J. Lipid Res.* **1987**, *28*, 464.
[10] R. A. Awl, E. N. Frankel, D. Weisleder, *Lipids* **1989**, *24*, 866.
[11] J. N. Jacobs, V. E. Shashona, A. Campbell, R. J. Baldessarini, *J. Med. Chem.* **1985**, *28*, 106.
[12] D. Buchnea, *Lipids* **1971**, *6*, 734.
[13] B. Yang, J. Chen, *J. Am. Oil Chem. Soc.* **1991**, *68*, 980.
[14] K. Aitzetmüller, M. Grönheim, *J. High Res. Chromatogr.* **1992**, *15*, 219.

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