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Synthesis of keto- and hydroxydienoic compounds from linoleic acid

Dmitry V. Kuklev[a,*], William W. Christie[b], Thierry Durand[c], Jean Claude Rossi[c], Jean Pierre Vidal[c], Sergey P. Kasyanov[a], Valery N. Akulin[a], Vladimir V. Bezuglov[d]

[a]*Pacific Research Institute of Fisheries and Oceanography (TINRO), Schevchenko, 4, Vladivostok, 690600, Russia*
[b]*Department of Chemistry, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*
[c]*Laboratoire de Chimie des Médiateurs et Physicochimie des Intéractions Biologiques associé au C.N.R.S., Université Montpellier I, Faculté de Pharmacie, 15 Av. Ch. Flahault, F-34060 Montpellier, France*
[d]*Department of PG and LT, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, The Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10; 117871 GSP 7 Moscow, Russia*

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Abstract

A convenient preparative method has been developed for the synthesis of hydroxydienoic (9-HODE and 13-HODE) and ketodienoic compounds (9-KODE and 13-KODE) from natural linoleic acid. Methyl linoleate was treated with 1.25 eq. of *m*-chloroperbenzoic acid in alcoholic solution, giving a mixture of mono-epoxides (yield 60%), that was treated with a solution of HBr in MeOH to yield a mixture of the bromohydrins (yield 92%). The last was oxidized by Jones reagent to a mixture of bromoketones (yield 64%) and the mixture obtained was dehydrobrominated by DBU to produce a mixture of ketodienoic compounds (yield 94%). Reduction of the ketodienoic compounds by KBH_4 in MeOH led to the corresponding hydroxydienoic (9-HODE and 13-HODE) methyl esters (yield 83%). The synthetic approach described is simple and gives reliable results. The keto- and hydroxy fatty acids obtained were characterized thoroughly by TLC, HPLC, UV, FT-IR, ^{1}H-, ^{1}H^{1}H- and ^{13}C-NMR. © 1997 Elsevier Science Ireland Ltd.

Keywords: Linoleic acid; Lipid peroxidation; Ketodienoic compounds; 9-Hydroxy-(10*E*,12*Z*)-octadecadien-1-oic acid (9-HODE); 13-Hydroperoxy-(9*Z*,11*E*)-octadecadien-1-oic acid (13-HODE); Synthesis; NMR spectroscopy; Mass spectrometry; High performance liquid chromatography

* Corresponding author.

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1. Introduction

Unsaturated fatty acids (UFAs) with a 1,4-*cis*,*cis*-pentadiene structural element (pentadienoic acids, PD-acids[1] are substrates for regio- and stereospecific addition of molecular oxygen, catalysed by lipoxygenases (Yamamoto, 1992), that result in the formation of hydroperoxides. One of the most abundant unsaturated acid in cellular systems that forms lipoxygenase products is (9*Z*,12*Z*)-octadecadien-1-oic acid (linoleic acid, LA) that can be referred to as mono-PD-acid. Via the established mechanism of enzymatic lipid peroxidation by lipoxygenases (Egmond et al., 1973), linoleic acid can yield products oxygenated in position 9 or 13 giving 9-hydroperoxy-(10*E*,12*Z*)-octadecadien-1-oic and (9-HPODE) and 13-hydroperoxy-(9*Z*,11*E*)-octadecadien-1-oic acid (13-HPODE) (Fig. 1). These primary products of peroxidation of linoleic acid can be reduced in the cells (e.g. under catalysis by glutathione-dependent peroxidases (Tappel et al., 1982; Bryant et al., 1983)) to the more stable hydroxy derivatives 9-hydroxy-(10*E*,12*Z*)-octadecadien-1-oic acid (9-HODE) or 13-hydroxy-(9*Z*,11*E*)-octadecadien-1-oic acid (13-HODE), respectively. Moreover, under specific conditions 9-HPODE and 13-HPODE can be transformed by lipoxygenase to ketodienoic compounds namely 9-keto-(10*E*,12*Z*)-octadecadien-1-oic acid (9-KODE) or 13-keto-(9*Z*,11*E*)-octadecadien-1-oic acid (13-KODE) (Vioque and Holman, 1962; Kühn et al., 1991), respectively. The hydroxydienoic and ketodienoic derivatives are the most common secondary products of lipid peroxidation derived from linoleic acid.

It should be emphasized that the secondary products have pronounced biological activity. Among the different biological responses (Spector et al., 1988; Needleman et al., 1986), chemotaxis (Henricks et al., 1991), hyperproliferative effects (Tsukada et al., 1986; Postoak et al., 1990), regulation of phospholipase activity (Chang et al., 1985) and regulation of cell adhesion (Honn et al., 1988; Honn et al., 1989) have been reported. The secondary products can be incorporated into phospholipids (Brezinski and Serhan, 1990), and by this means they can be involved in regulation of biological membrane states.

The main goals of the present work were to develop a preparative synthetic route to both the secondary products of lipid peroxidation of linoleic acid, and to perform their characterization[2].

2. Experimental

2.1. Materials and equipment

Acetone, benzene, ether, *n*-, *iso*- and *c*-hexane were distilled over P_2O_5, ethanol, *iso*-propanol and methanol were distilled from metallic Na, and methylene chloride was stored over $CaCl_2$ and DBU over CaH_2 in vacuo. All chemicals used were of analytical reagent grade.

The TLC analysis was performed with the use of precoated TLC plates (DC-Alufolien Kieselgel 60 F_{254}, Merck, Germany). The plates were developed by spraying with a 5% solution of phosphomolybdic acid in EtOH and heating at 110°C for about 2 min. Visualisation of the spots was done under a UV lamp at 254 nm. Preparative column separation and purification were performed with

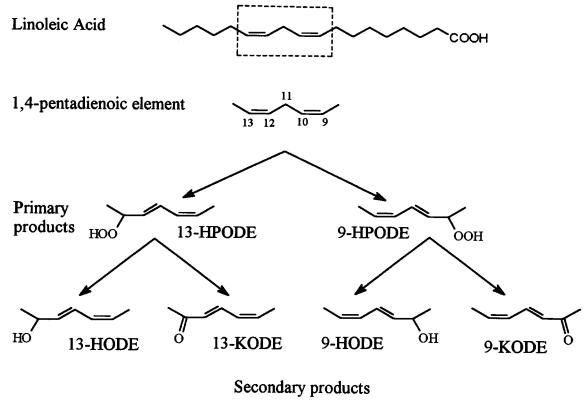

Fig. 1. Peroxidation of linoleic acid.

[1] (The term 'PD-acids' was introduced to encompass the polyenoic fatty acids bearing the pentadienoic fragment(s) (Kuklev et al., 1996).

[2] For the preliminary communications see in (Kuklev et al., 1996).

Kieselgel 63–200 μm (70–230 mesh ASTM) (Merck, Germany).

For high performance liquid chromatography (HPLC) (analytical and semi-preparative), a Waters Model 510 pump (Millipore, USA) with a Waters 490 UV detector were used. The columns and systems applied for each separation are described below.

UV spectra were recorded on a Varian DMS 90 UV/visible spectrophotometer (Varian, Australia) instrument at a scanning rate of 20 nm/min from 700 down to 200 nm in methanol or ethanol.

IR-spectra of films of the mixtures and individual compounds were recorded on a Perkin Elmer FT-IR 1760 instrument (Perkin Elmer, Germany).

^{1}H-(400, 500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker WM500 instrument (Bruker, Germany) at 25°C, and the chemical shifts (δ, ppm) are presented relative to an internal standard $(CH_3)_4Si$ for solutions in $CDCl_3$. All signal assignments were carried out with proton-proton decoupling in support of experiments.

Mass spectra (EI) were recorded on a Jeol DX300 (Jeol, Japan) instrument at 70 eV. Gas chromatography-mass spectrometry was carried out with a Kratos 8/90 double-focusing magnetic sector instrument, with electron-impact ionization (EI, at 70 eV), attached to a Varian 2700 gas chromatograph (Varian Ltd., UK) with a WCOT fused silica (25 m × 0.25 mm) column coated with OV-1 (Supelco, USA) bonded phase. All mass spectra were obtained with temperature programming from 100 up to 300°C at a heating rate of 5°C/min.

2.2. Synthesis of epoxides of linoleic Acid (**2**,**3**)

With stirring, a solution of 3.67 g (1.25 eq.) of *m*-chloroperbenzoic acid in 50 ml of absolute ethanol was added dropwise to a solution of 5.0 g of linoleic acid methyl ester (Fig. 2, **1**) in 50 ml absolute ethanol. The reaction mixture was allowed to stand for 1 h and then 300 mg of Na_2SO_3 was added. After vigorous stirring, the solvent was evaporated under vacuum, and the residue was taken into a minimal volume of hexane; the resulting solution was chromatographed on silica gel with elution by a gradient ($0 \rightarrow 25\%$) of diethyl ether in *iso*-hexane. This yielded 3.44 g (65%) of a mixture of isomeric epoxy esters in the form of a colourless mobile oil. R_f 0.38 (Fig. 3) – methyl ester of 12,13-epoxy-(9*Z*)-octadecen-1-oic acid (**2**), R_f 0.33 – methyl ester of 9,10-epoxy-(12*Z*)-octadecen-1-oic acid (**3**). From the reaction mixture 1.2 g of the parent acid was isolated. The yield of the epoxides was 86% in terms of conversion. Diepoxides were present only in trace amounts. The mixture of **2** and **3** was used further without separation. UV spectroscopy of a mixture of **2** and **3** showed no maximum at $\lambda > 206$ nm. IR, cm^{-1}: 2928 s, 2856 s, 1741 s, 1460 m., 136 m, 1379 m, 1248 w, 1197 m, 1171 m, 1016 w.

For structure assignment the individual epoxides were separated by HPLC (column: Hypersil H3 (250 × 4.6 mm), 3 μm; the mobile phase was *iso*-hexane/*i*-propanol (99.6:0.4, v/v), at a flow-rate of 1 ml/min and with UV-detection at 206 nm). The compound with $k' = 0.62$ was analysed by GC-MS in the form of the trimethylsilyl derivatives of the corresponding chlorohydrins (Section 2.3) and gave fragmentation as follows (most prominent ions, presumed structures of the ions): 418 [M], 299 [M – $ClCHC_5H_{11}$], 221 [(TMSO)$CHCH(Cl)C_5H_{11}$], 173 [$CH(TMSO)\ C_5H_{11}$] and was recognized as (**2**). The compound with $k' = 0.8$ (processed as described above) gave fragmentation as follows (most prominent ions, presumed structures of the ions): 418 [M], 307 [M – $C_5H_{11}(CH{=}CH)CH_2$], 259 [M – $C_5H_{11}(CH{=}CH)CH_2CH(Cl)$], 213 [$C_5H_{11}(CH{=}CH)CH_2CH(OTMS)$] and was recognized as (**3**).

2.3. Trimethylsilyl (TMS) derivatives of chlorohydrins of the fatty acids for mass-spectrometric analysis

A sample of the methyl ester of an epoxy acid (1–3 mg) was dissolved in 200 mkl of dry pyridine, 50 mkl of trimethylchlorosilane was added, and the mixture was kept at 50°C for 2 h. After cooling, it was evaporated in a stream of nitrogen, and the dry residue was taken into 3 ml of *i*-hexane and filtered through cotton wool. The TMS ethers of the chlorohydrins of the fatty acid methyl esters so obtained had R_f 0.9 (*n*-hexane-ether (80:20, v/v).

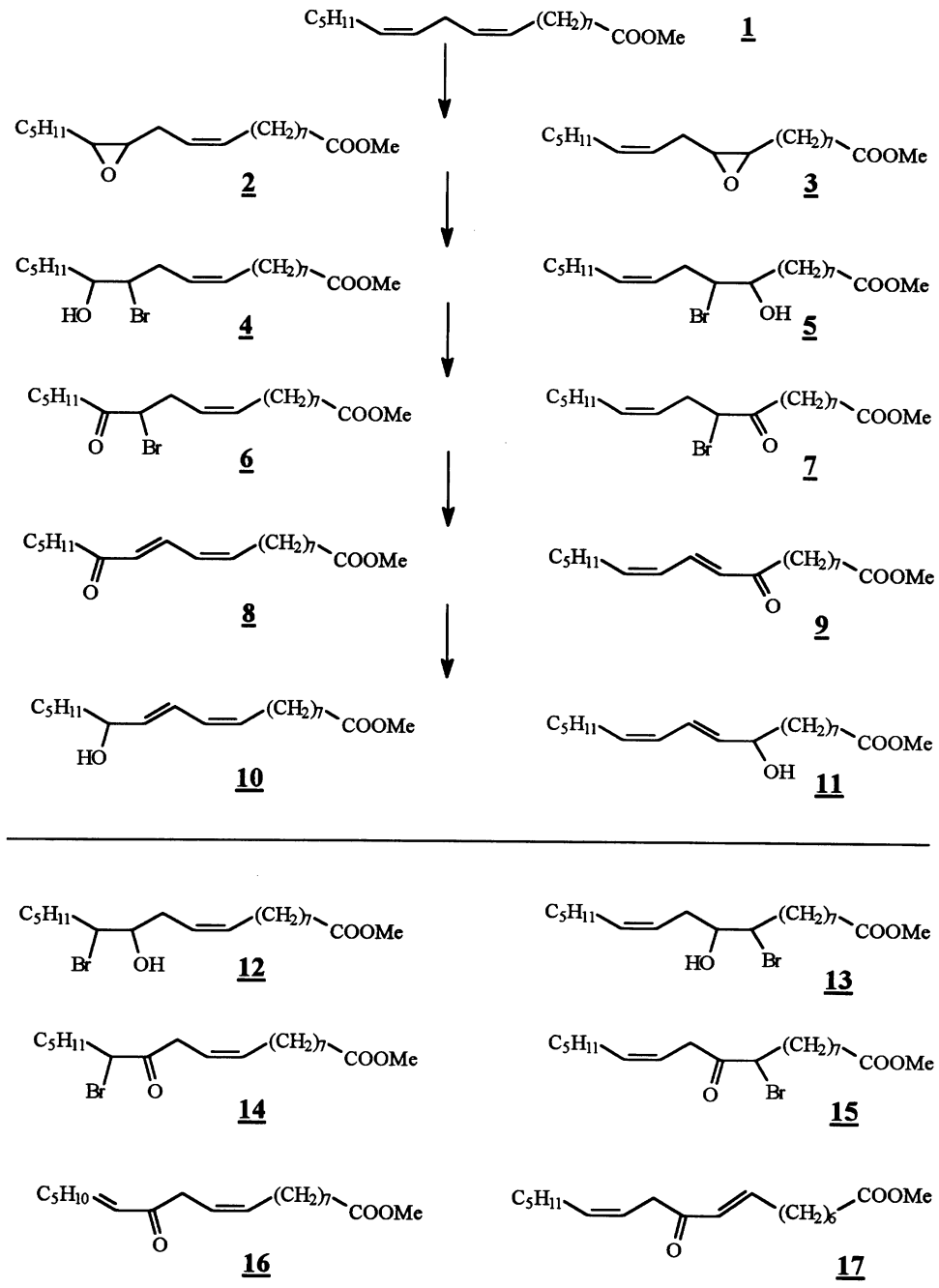

Fig. 2. Synthetic scheme.

2.4. Synthesis of isomeric bromohydrins (4,5,12,13)

A solution of 2 ml of AcBr in 20 ml of MeOH was prepared immediately before the use. One gram of the mixture of epoxides (**2**,**3**) was dissolved in this solution at −10°C and the mixture was kept for 1 h at this temperature. The reaction mixture was diluted with 20 ml of water and extracted with hexane (3 × 50 ml). The extract was washed with a saturated solution of NaCl (100 ml) and dried over anhydrous Na_2SO_4. The dry extract was evaporated under vacuum, the residue was dissolved in a minimal volume of hexane and subjected to column chromatography on 20 g silica gel with elution by a gradient (10 → 50%) of diethyl ether in *i*-hexane. This gave 1.1 g (87%) of a mixture of the isomeric bromohydrins in the form of a colourless mobile oil. UV spectroscopy of the mixture of **4**,**5**,**12** and **13** showed no maximum at $\lambda > 206$ nm. IR, cm^{-1}: 3468 s wide, 2928 s, 2856 s, 1740 s, 1460 m, 1437 m, 1377 w, 1248 w, 1199 m, 1173 m, 1077 w. R_f 0.13–0.24 (Fig. 3). The compounds (**4**,**5**,**12**,**13**) synthesised were used further without separation.

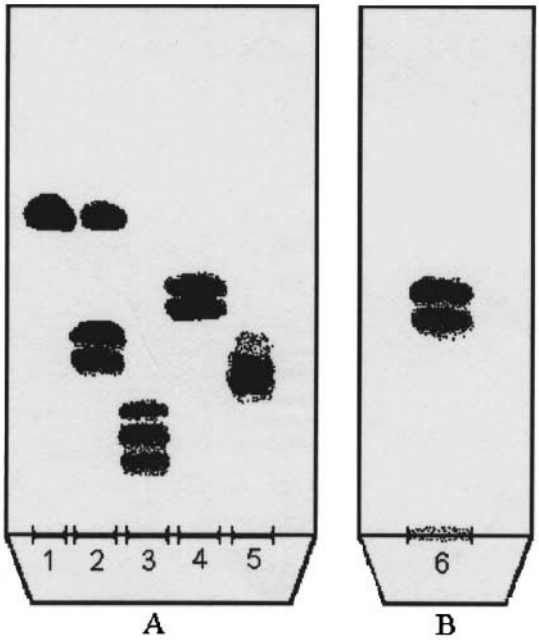

Fig. 3. Thin layer chromatography traces of the reaction mixtures. Plate A: The developing system used was a mixture of *n*-hexane:diethyl ether (80:20, v/v) at +20°C. The plate was visualized by spraying with phosphomolybdic acid in EtOH and heating at 110°C for about 2 min. Stripe 1: Methyl linoleate (**1**) R_f 0.6. Stripe 2: The reaction mixture of the epoxidation to the mixture of epoxides (**2**) and (**3**). The spot with R_f 0.6 was produced by the remains of the parent methyl linoleate; the spot with R_f 0.38 was produced by the 12,13-epoxy derivative (**2**); the spot with R_f 0.33 was produced by the 9,10-epoxy derivative (**3**). Stripe 3: The reaction mixture of the bromohydrins synthesis; the diffuse spot with R_f 0.13–0.24 was produced by the four bromohydrins(**4**,**5**,**12**,**13**). Stripe 4: The reaction mixture of oxidation of the bromohydrins (**4**,**5**,**12**,**13**) to bromoketones (**6**,**7**,**14**,**15**). R_f 0.42–0.50. Stripe 5: Ketodienoic compounds (**8**,**9**); the spot with R_f 0.32 was produced by the 13-keto-isomer (**8**) whereas the spot with R_f 0.28 was produced by the 9-keto isomer (**9**). Plate B: The developing system used was a mixture of *n*-hexane:diethyl ether (50:50, v/v) at +20°C. The plate was visualized by spraying with phosphomolybdic acid in EtOH and heating at 110°C for about 2 min. Stripe 6: Mixture of methyl esters of the required HDCs: R_f 0.46: 13-HODE (**10**). R_f 0.40: 9-HODE (**11**).

2.5. Synthesis of isomeric bromoketones (6,7,14,15)

A solution of the 1.10 g of the bromohydrins (**4**,**5**,**12**,**13**) in 50 ml of acetone was cooled to −40°C and 2.0 ml of standard Jones reagent were added. Then the reaction temperature was allowed to increase to −15°C, and the reaction mixture was kept at this temperature for 40 min, after which 10 ml of *iso*-propanol were added and the reaction mixture was warmed up to room temperature, diluted with 50 ml of water and extracted with hexane (3 × 100 ml). The extract was washed with a saturated solution of NaCl (100 ml) and dried over anhydrous Na_2SO_4. The dried extract was evaporated in vacuo and the residue was taken into a minimal volume of hexane and subjected to column chromatography on 20 g silica gel with elution by a gradient (0 → 20%) of diethyl ether in *i*-hexane. This gave 700 mg (64%) of a mixture of the isomeric bromoketones (**6**,**7**,**14**,**15**) in the form of a bright yellow mobile oil with a characteristic odour. UV spectroscopy of the mixture of **6**,**7**,**14** and **15** showed no maximum at $\lambda > 206$ nm. IR, cm^{-1}: 2928 s, 2856 s, 1739 s, 1599 w, 1460 m, 1435 m, 1363 w, 1248 w, 1197 w, 1170 w, 1019 w. R_f 0.42–0.50 (Fig. 3).

The compounds synthesised were used further without separation.

2.6. Synthesis of isomeric conjugated ketocompounds (**8**,**9**,**16**,**17**)

Six-hundred-and-fifty milligrams of the bromoketones (**6**,**7**,**14**,**15**) were dissolved into 50 ml of dry benzene and 1 ml of DBU (4 eq.) was added. The reaction mixture was kept for 30 min at room temperature, then it was filtered through 10 g of silica gel, and the cartridge was washed with 100 ml of dry benzene. The filtrate was evaporated in vacuo, giving 481 mg (94%) of a mixture of the isomeric conjugated ketones (**8**,**9**,**16**,**17**) in the form of a bright yellow oil. R_f 0.32–0.12 (*n*-hexane-diethyl ether (80:20, v/v) (diffuse spot)). The dry oil obtained was taken into a minimal volume of *i*-hexane and subjected to column chromatography on 25 g silica gel with elution by a gradient ($0 \rightarrow 20\%$) of diethyl ether in *i*-hexane. This gave 220 mg of a mixture of the target ketodienoic compounds (**8**) and (**9**) in the form of a viscous colourless oil. R_f 0.28–0.32 (Fig. 3) (diffuse spot). The ketodienoic compounds (**8**) and (**9**) were separated by semi-preparative HPLC (column: Hibar® 5 μm, Si60 (10×250 mm) (Merck, Germany), eluent: *n*-hexane:*iso*-propanol (1000:2, v/v) at a flow rate of 6 ml/min). Methyl ester of 13-oxo-(9*Z*,11*E*)-octadecadien-1-oic acid (13-KODE methyl ester) (**8**). Colorless oil, R_f 0.32 (Fig. 3). HPLC: $k' = 6.57$; (Fig. 4, peak I). UV (MeOH): $\lambda_{max} = 278$ nm, $\epsilon = 20\,330$. IR (film, cm^{-1}): 2930, 2856, 1740, 1688, 1666, 1630, 1590, 1461, 1436, 1411, 1363, 1260, 1196, 1173, 1085, 996, 961, 870, 799, 725. ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 0.89 (t, 3H, H-18; $J_{18,17}$ 6.9), 1.32 (m, 10H, H-4,5,6,16,17), 1.43 (m, 2H, H-3), 1.59 (m, 4H, H-7,15), 2.28 (t, 2H, H-2; $J_{3,2}$ 7.5), 2.28 (m, 2H, H-8), 2.55 (t, 2H, H-14, $J_{14,15}$ 7.5), 3.67 (s, 3H, $C\underline{H}_3OOC$), 5.9 (ddt, 1H, H-9, $J_{9,10}$ 11.1, $J_{9,8}$ 7.8, $J_{9,11}$ 0.9), 6.1 (ddd, 1H, H-10, $J_{10,9}$ 11.1, $J_{10,11}$ 10.6, $J_{10,12}$ 0.9), 6.14 (dd, 1H, H-12, $J_{12,11}$ 15.4, $J_{12,10}$ 0.9), 7.43 (ddd, 1H, H-11, $J_{11,12}$ 15.4, $J_{11,10}$ 11.1, $J_{11,9}$ 0.9) (Fig. 5). ^{13}C-NMR (125 MHz, $CDCl_3$, δ): 13.8 (C-18), 22.5 (C-17), 24.2, 24.9, 28.4, 29.2, 29.2, 28.9, 29.2, 32.0 (C-3,4,5,6,7,8,15,16), 34.1 (C-2), 41.1 (C-14), 51.4 (^{C}H$_3$O), 127.0 (C-10), 129.4 (C-12), 136.9 (C-9), 142.4 (C-11), 173.2 (C-1), 202.1 (C-13) (Fig. 6). ^{1}H-^{1}H COSY (400 MHz, $CDCl_3$) (Fig. 5): the ^{1}H-^{1}H COSY spectrum of **8** clearly showed the following connectivities (H-H): 2-3, 3-4, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 14-15, 15-16, 17-18. EIMS of the reduced ($NaBH_4$, H_2 over PtO_2) methyl ester and TMS-ether, m/z (I%): 371 $[M - Me]^+$ (7%), 315 $[M - C_5H_{11}]^+$ (100%), 173 $[TMSOCHC_5H_{11}]^+$ (10%).

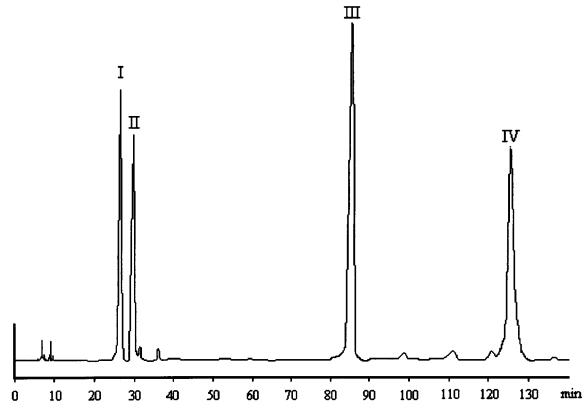

Fig. 4. HPLC analysis of standard ketodienoic compounds (I — **8**, II — **9**) and hydroxydienoic compounds (III — **10**, IV — **11**). Conditions: column Zorbax-SIL (250×4.6 mm), 5 μm, SiO_2; the mobile phase was a mixture of *n*-hexane with *i*-propanol (1000:3, v/v), at a flow-rate of 0.9 ml/min, UV-detection at 206 nm).

Methyl ester of 9-oxo-(10*E*,12*Z*)-octadecadien-1-oic acid (9-KODE methyl ester) (**9**): Colourless oil, TLC: R_f 0.28 (Fig. 3). HPLC: $k' = 7.57$, (Fig.

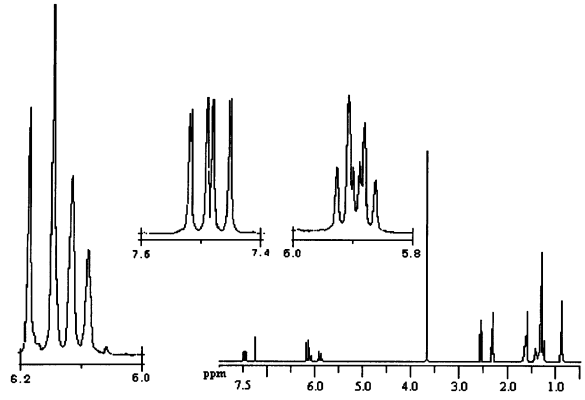

Fig. 5. ^{1}H-NMR and ^{1}H^{1}H-NMR spectra of methyl ester of 13-oxo-(9*Z*,11*E*)-octadecadien-1-oic acid (**8**).

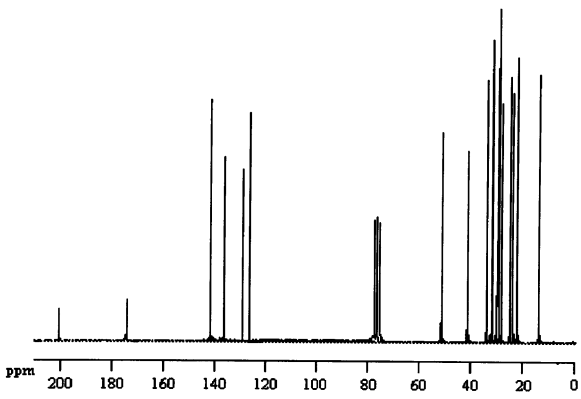

Fig. 6. ^{13}C-NMR spectrum of methyl ester of 13-oxo-(9*Z*,11*E*)-octadecadien-1-oic acid (*8*).

4, peak II). UV (MeOH): $\lambda_{max} = 278$ nm, $\epsilon = 18\,700$. IR (film) cm^{-1}: 2929, 2856, 1740, 1688, 1665, 1631, 1590, 1462, 1436, 1411, 1364, 1260, 1196, 1173, 1085, 1110, 997. ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 0.89 (t, 3H, H-18; $J_{18,17}$ 6.9), 1.32 (m, 10H, H-4,5,6,16,17), 1.43 (m, 2H, H-3), 1.59 (m, 4H, H-7,15), 2.30 (t, 2H, H-2; $J_{3,2}$ 7.5), 2.33 (m, 2H, H-14), 2.55 (t, 2H, H-8, $J_{7,8}$ 7.4), 3.67 (s, 3H, C$\underline{H}_3$OOC), 5.92 (ddt, 1H, H-13, $J_{13,12}$ 11.1, $J_{13,14}$ 7.9, $J_{13,11}$ 0.9), 6.13 (ddd, 1H, H-12, $J_{12,13}$ 11.1, $J_{12,11}$ 10.25, $J_{12,10}$ 0.9), 6.14 (dd, 1H, H-10, $J_{10,11}$ 15.1, $J_{10,12}$ 0.9), 7.47 (ddd, 1H, H-11, $J_{11,10}$ 15.1, $J_{11,12}$ 10.25, $J_{11,13}$ 0.9). ^{13}C-NMR (125 MHz, $CDCl_3$, δ): 13.9 (C-18), 22.5 (C-17), 24.1, 24.9, 28.3, 29.1, 29.1, 29.1, 29.3, 31.5 (C-3,4,5,6,7,8,15,16), 34.1 (C-2), 41.1 (C-14), 51.5 (C$\underline{H}_3$O), 127.0 (C-10), 129.4 (C-12), 136.9 (C-13), 142.4 (C-11), 174.2 (C-1), 201.1 (C-9). ^{1}H-^{1}H COSY (400 MHz, $CDCl_3$) (Fig. 5): the ^{1}H-^{1}H COSY spectrum of **9** clearly showed the following connectivities (H-H): 2-3, 3-4, 6-7, 7-8, 10-11, 11-12, 12-13, 13-14, 14-15, 15-16, 17-18. EIMS of the reduced ($NaBH_4$, H_2 over PtO_2) methyl ester and TMS-ether, *m*/*z* (I%): 371 [M – Me]$^+$ (10%), 259 [M – C_9H_{19}]$^+$ (100%), 229 [TM-SOCHC$_9$H$_{19}$]$^+$ (8%).

*2.7. Synthesis of isomeric hydroxydienoic compounds (**10**,**11**)*

A solution of 283 mg of the ketodienoic compounds in 50 ml MeOH was cooled down to -50°C and 100 mg of KBH_4 (2 eq.) were added. Then the reaction temperature was allowed to increase up to -10°C during 2 h, after which 10 ml of a saturated solution of NH_4Cl were added, the resulting solution was diluted with water (50 ml), and extracted with *n*-hexane (3 × 100 ml). The extract was washed with a saturated solution of NaCl (100 ml) and dried over anhydrous Na_2SO_4. The dried extract was evaporated in vacuo and the residue was taken into a minimal volume of hexane and subjected to column chromatography on 20 g silica gel with elution by a gradient (10 → 70%) of diethyl ether in *i*-hexane. This gave 236 mg (83%) of a mixture of the isomeric hydroxy dienoic compounds in the form of a colourless mobile oil. R_f 0.4–0.46 (Fig. 3). The hydroxy derivatives synthesised were analysed by HPLC (Fig. 4) and separated by semi-preparative HPLC (conditions: column Hibar® 5 μm, Si60 (10 × 250 mm) (Merck, Germany), mobile phase: *n*-hexane:*iso*-propanol (1000:4, v/v); at a flow rate of 6 ml/min). Methyl ester of 13-hydroxy-(9*Z*,11*E*)-octadecadien-1-oic acid (13-HODE methyl ester). TLC: $R_f = 0.46$ (Fig. 3). UV (EtOH): $\lambda_{max} = 233$ nm, $\epsilon = 18\,200$, HPLC: $k' = 23.43$ (Fig. 4, peak III). ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.86 (t, 3H, H-18), 1.2–1.7 (m, 18H, CH_2), 2.14 (m, 2H, H-8), 2.27 (t, 2H, H-2, $J_{2,3}$ 7.6), 3.64 (t, 3H, CH_3OOC), 4.13 (m, 1H, H-13), 5.40 (ddt, 1H, H-9, $J_{9,10}$ 11.4, $J_{9,8}$ 7.6, $J_{9,12}$ 1), 5.64 (dd, 1H, H-12, $J_{12,11}$ 15.4, $J_{12,13}$ 6.5), 5.94 (dd, 1H, H-10, $J_{10,11}$ 10.4 $J_{9,10}$ 11.5), 6.45 (ddd, 1H, H-11, $J_{11,12}$ 15.4, $J_{11,10}$ 10.4, $J_{11,9}$ 1). EIMS of TMS-ether on hydroxyl group, *m*/*z*: 382 [M]$^+$, 311 [M – C_5H_{11}]$^+$, 225 [M – $(CH_2)_7$COOMe]$^+$, 173 [CH(TMSO)C_5H_{11}]$^+$. Methyl ester of 9-hydroxy-(10*E*,12*Z*)-octadecadien-1-oic acid (9-HODE methyl ester). TLC: $R_f = 0.40$ (Fig. 3). UV (EtOH): $\lambda_{max} = 233$ nm, $\epsilon = 17\,200$; HPLC: $k' = 34.86$, (Fig. 4, peak 4). ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 0.86 (t, 3H, H-18, $J_{18,17}$ 7.5), 1.2–1.7 (m, 18H, CH_2), 2.15 (m, 2H, H-14), 2.27 (t, 2H, H-2, $J_{2,3}$ 7.6), 3.64 (t, 3H, C$\underline{H}_3$OOC), 4.12 (m, 1H, H-9), 5.42 (ddt, 1H, H-13, $J_{13,12}$ 11.2, $J_{13,14}$ 7.8, $J_{13,11}$ 1), 5.63 (dd, 1H, H-10, $J_{10,11}$ 15.4, $J_{10,9}$ 6.8), 5.94 (dd, 1H, H-12, $J_{12,13}$ 11.2 $J_{12,11}$ 10.6), 6.46 (ddd, 1H, H-11, $J_{11,10}$ 15.4, $J_{11,12}$ 10.6, $J_{13,11}$ 1). EIMS of TMS-ether on hydroxyl group, *m*/*z*: 382 [M]$^+$, 311 [M – C_5H_{11}]$^+$, 259 [M – C_5H_{11} (CH═CH)$_2$]$^+$, 225 [C_5H_{11}(CH═CH)$_2$CH(TMSO)]$^+$.

3. Results and discussion

Our goal was to develop a practical approach for synthesis of ketodienoic and hydroxydienoic compounds from natural pentadienoic UFAs (Fig. 1). As the first UFA to be converted to the oxygenated products we took linoleic acid, which is a mono-PD-acid and contains the only pentadienoic element at the ninth position of its chain.

The first step in our synthetic scheme was synthesis of the mixture of mono-epoxides (**2**,**3** – Fig. 2) by the Prileschajew reaction. We used *m*-chloroperbenzoic acid in ethanol for the purpose, as it gives stable high yields (as can be seen in the literature (VanRollins et al., 1989; VanRollins, 1990)) and has been used by us for synthesis of monoepoxides from PUFAs (Kuklev et al., 1993). Carrying out the reaction in a polar protonic solvent was essential here because of the lower activity of *m*-chloroperbenzoic acid in such solvents (Prileschajewa, 1974). The use of an excess of the reagent (more than 1.25 eq.) leads to increasing amounts of byproducts, even under the mild conditions described. The epoxides synthesised (**2**,**3**) were separated from the remains of the parent methyl linoleate (**1**) and from traces of diepoxide by column chromatography. In terms of conversion the yield was 86% at this stage.

The second step was cleavage of the monoepoxides (**2**,**3**) to bromohydrins (**4**,**5**,**12**,**13**) with the use of HBr/MeOH at low temperature (-40 to -20°C). When we used other reagents, e.g. a saturated solution of KBr/AcOH/THF (Corey et al., 1980) or a two-phase system of 32% HBr/*n*-heptane (Kuklev et al., 1991) (that we developed for cleavage 4,5- and 5,6-epoxides of PUFA), the yields did not exceeded 50–60% due to byproduct formation (data not shown). Cleavage by HBr/MeOH at -30°C proceeded smoothly and gave reliable high yields of more than 90%, almost without byproducts.

The key point in our synthetic strategy was oxidation of the bromohydrins (**4**,**5**,**12**,**13**) to bromoketones (**6**,**7**,**14**,**15**). This step can be viewed as a 'protection' of the hydroxyl group against elimination of a proton by DBU. Using different protective groups (acetate, benzoate, trimethylsilyl) leads to decreasing of the reaction rate and to byproducts as a result of double elimination of HBr, first, and ROH as the second step (where R is acetate, benzoate or TMS) (data not shown). In the case of bromoketones whose reactivity is very high toward to dehydrobromination to yield conjugated ketones, there is no theoretical possibility of producing the products of double elimination.

From another standpoint, in our strategy we had synthesised ketodienoic compounds first and then we reduced them to obtain allylic alcohols. It is important to point out the lability of allylic alcohols toward oxidation. In contrast, α-bromoalcohols (**4**,**5**,**12**,**13**) are sufficiently stable to oxidation and can be transformed to α-bromoketones (**6**,**7**,**14**,**15**) in a yield of more than 60% (by Jones reagent) without major byproducts.

Dehydrobromination of bromoketones (**6**,**7**, **14**,**15**) to conjugated ketones (**8**,**9**,**16**,**17**) by the action of DBU in dry benzene went smoothly and with high yields. The ketodienoic compounds (**8**,**9**) and ketomonoenoic compounds (**16**,**17**) had a pronounced difference in chromatographic mobility and could be separated by preparative column chromatography. We focused our attention on natural ketodienoic compounds (**8**,**9**) and do not present data on ketomonoenoic derivatives (**16**,**17**). It should be stressed that the double bond newly created has the *trans*-configuration, as can be seen from the ^{1}H- and ^{13}C-NMR data (Figs. 5 and 6). An additional confirmation of the stereochemistry of the products came from the IR-spectra where a band of absorbtion at 965 cm^{-1} was characteristic for a *trans*-double bond. As can be seen from the ^{1}H-NMR spectrum (Fig. 5), the proton at C-11 (7.47 ppm) produces a characteristic signal in the form of ddd with the coupling constants $J_{11,10}$ 15.1 Hz (*trans*-double bond), $J_{11,12}$ 10.25 Hz (single bond) and $J_{11,13}$ 0.9 Hz (far constant in conjugated system of double bonds). It should be emphasized that possibility to see the far constant with a value of only 0.9 Hz can be accepted as a confirmation of purity of the ketodienoic compounds synthesised. At the present time there is no reliable information about the ^{1}H-NMR characteristics of natural 9-KODE and 13-KODE. To clarify this point and to prove our signal assignment we performed a two-dimensional ^{1}H^{1}H-NMR-experiment (Fig. 5). It is

clearly seen from the spectrum that there are coupling constants between the proton at 7.47 ppm and protons at 6.13 ppm and 6.14 ppm, and these are supported by the nature of multiplets of this signals (Fig. 5); the signal can be assigned to protons at C-11, C-12 and C-10 respectively. In the ^{13}C-NMR (Fig. 6) spectrum, there are four signals of the *cis*,*trans*-conjugated system at 127.0 (C-10), 129.4 (C-12), 136.9 (C-13), 142.4 (C-11). We did not observe an explainable difference in ^{1}H- and ^{13}C-NMR spectra of 9-KODE and 13-KODE, so we present only spectra of 13-KODE. No difference in the FT-IR-spectra of 9-KODE and 13-KODE was found, especially in the fingerprint area. The identity of the ketodienoic compounds (**8**,**9**) to the natural ones can be seen from comparing their UV and IR-data (Vioque and Holman, 1962; Kühn et al., 1991).

The reduction of the ketodienoic compounds to the required hydroxy fatty acids (**10**,**11**) was performed by the action of potassium borohydride in methanol at −50°C. Using sodium borohydride in methanol (or ethanol) as a reagent under the same conditions led to degradation of the *cis*-*trans* conjugated system of double bonds as judged by UV-spectra and by HPLC analysis of the processed reaction mixtures (Figs. 7 and 8). The expected conjugated hydroxy fatty acids were

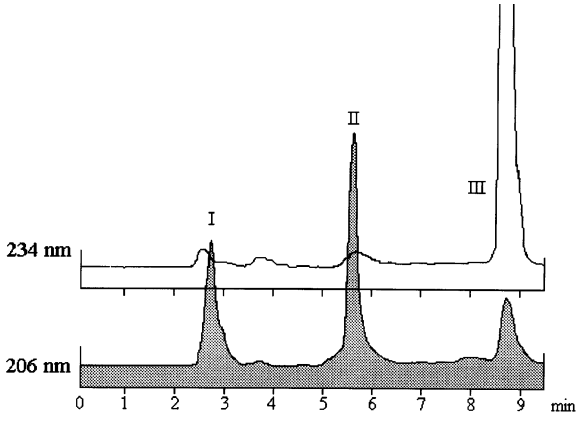

Fig. 7. RP-HPLC analysis of the processed reaction mixture of the reduction of the ketodienoic compounds (**8**,**9**) by $NaBH_4$ in methanol at −30°C (Column: Spherisorb ODS2 5 μm (250 × 4.6 mm). Mobile phase: acetonitrile:water (90:10, v/v); flow-rate of 0.7 ml/min and with UV-detection at 206, 234 nm).

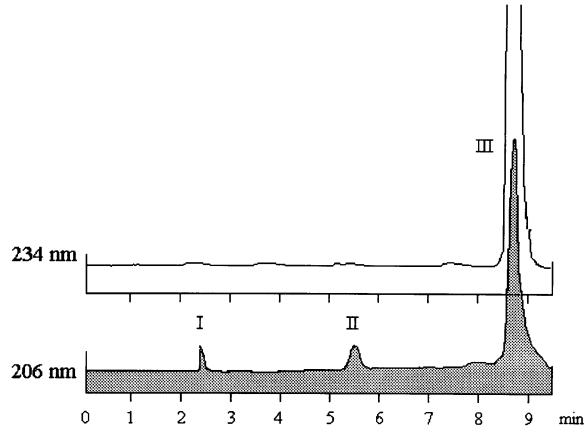

Fig. 8. RP-HPLC analysis of the processed reaction mixture of the reduction of the ketodienoic compounds (**8**,**9**) by KBH_4 in methanol at −30°C (Column: Spherisorb ODS2 5 μm (250 × 4.6 mm). Mobile phase: acetonitrile:water (90:10, v/v); flow-rate of 0.7 ml/min and with UV-detection at 206, 234 nm).

only found at trace levels, as shown Fig. 7 (peak III – unseparated mixture of 9- and 13-HODE, major peak by detection at 233 nm, but minor component at 206 nm), judging by the absorbtion at 206 nm. With the use of potassium borohydride we succeeded in the reduction of the ketodienoic compounds (**8**,**9**) to the required conjugated hydroxy fatty acids (**10**,**11**) as shown in Fig. 8 (major peak III – unseparated mixture of 9- and 13-HODE, absorbing both at 206 nm and 234 nm as a major component). The hydroxy derivatives were isolated on a preparative scale by SP-HPLC and reliably characterized by UV, MS and ^{1}H-NMR (Section 2.7).

The synthetic approach developed is simple and gives reliable results. It is of particular interest as it gives access to both ketodienoic and hydroxy compounds. We intend to apply it to the synthesis of a wide range of oxidized derivatives from other fatty acids. This will give us a 'library' of hydroxy and ketodienoic compounds that will serve us as standards during biological investigations.

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