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Easy access to various natural keto polyunsaturated fatty acids and their corresponding racemic alcohols

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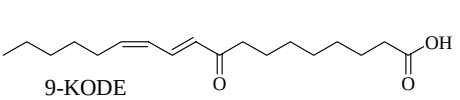
Abstract

Various optically active hydroxy derivatives of polyunsaturated fatty acids were easily oxidised to their corresponding keto derivatives using Dess-Martin periodinane. The reaction was run on the millimolar scale with good yields and without appreciable isomerisation of the surrounding double bonds. Reduction of these keto compounds to yield back the starting alcohols, but now as racemic mixtures, was also conducted using $CeCl_3$–$NaBH_4$, once again without noticeable modification of the stereochemistry of the double bonds. These reactions proved the usefulness of the chemoenzymatic access to oxylipins through the use of lipoxygenases with various regiospecificity, combined with chemical transformations of the formed hydro(pero)xides.

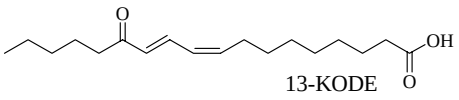
Keywords: Dess-Martin periodinane; Oxylipin; $CeCl_3$; Ketodiene; Soybean lipoxygenase; Barley seed lipoxygenase

1. Introduction

Recently, it has been shown (Vollenweider et al., 2000) that ketodienoic fatty acids (9-KODE and 13-KODE) accumulate after wounding and infection of *Arabidopsis thalliana* by *Pseudomonas syringae*. Infiltration of such compounds in the plant causes both the expression of the gene coding glutathion-*S*-transferase (*GST 1*) and cell death (Vollenweider et al., 2000).



9-KODE



13-KODE

It has been known for a long time that various metabolites of the so-called octadecanoid pathway, involving lipoxygenase as a key enzyme, are of primary importance in plant physiology (Feussner and Wasternack, 2002; Weber, 2002; Blée, 2002; Howe and Schilmiller, 2002). Such products like jasmonates, volatile aldehydes, fatty aldehydes and others are both involved in response to environmental stress and developmental cues. The role of ketodienes as well

Abbreviations: 9-HODE, 9-hydroxy-10*E*,12*Z*-octadecadienoic acid; 9-HOTE, 9-hydroxy-10*E*,12*Z*,15*Z*-octadecatrienoic acid; (Me-) 13-HODE, 13-hydroxy-9*Z*,11*E*-octadecadienoic acid (methyl ester); (Me-) 13-HOTE, 13-hydroxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (methyl ester); 9-KODE, 9-oxo-10*E*,12*Z*-octadecadienoic acid; 9-KOTE, 9-oxo-10*E*,12*Z*,15*Z*-octadecatrienoic acid; (Me-) 13-KODE, 13-oxo-9*Z*,11*E*-octadecadienoic acid (methyl ester); (Me-) 13-KOTE, 13-oxo-9*Z*,11*E*,15*Z*-octadecatrienoic acid (methyl ester); BSLOX, barley seed lipoxygenase; SBLOX, soybean lipoxygenase

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as their biosynthesis are important emerging areas in plant physiology.

In order to study the role of keto acids in plants, a general synthetic method is required, applicable to both linoleic and α-linolenic acids, on either positions 9 and 13 of these acids. A much important point is the control of the stereochemistry of the two double bonds conjugated to the keto functionality which should be *E*,*Z*.

We have developed recently a very simple chemoenzymatic synthesis of both 9- and 13-hydroxydienoic derivatives of linoleic and α-linolenic acid, using BSLOX for 9 oxygenation and SBLOX for 13 oxygenation, followed by triphenylphosphine reduction of the formed hydroperoxides (Iacazio et al., 1990; Martini et al., 1994; Martini et al., 1996; Martini and Iacazio, 1997). Key points of these transformations were the high fatty acid concentration used (up to 0.1 M), the high yield (∼80%, 1 g scale easily accessible) and the proper control of both the stereochemistry of the newly formed conjugated system and the regioselectivity of the insertion of the hydro(pero)xy functionality. This led to the synthesis of 9-HODE, 13-HODE, 9-HOTE and 13-HOTE with more than 95% isomeric purity and, although not relevant here, with at least 98% enantiomeric excess (*S* enantiomer). Based on these results it was therefore of interest to look for an oxidation system, able to generate keto acids from these easily accessible hydroxy acids, without affecting the stereochemistry of the conjugated dienoic system of such natural products.

During the course of this study, Koch et al. reported on the synthesis of 9-KOTE and 13-KOTE by the chemo-enzymatic approach described above (Koch et al., 2002). Lipoxygenation of α-linolenic acid was conducted by either tomato lipoxygenase (9-oxygenation) or SBLOX (13-oxygenation) and after reduction of the formed hydroperoxides, the ketodienes were obtained through oxidation of the allylic alcohols by Bobitt's reagent (Bobbit, 1998). The reaction was conducted on either 23 mg of 13-HOTE or 8.7 mg of 9-HOTE affording, respectively 13.3 mg of 13-KOTE (58% yield) and 6 mg of 9-KOTE (69% yield).

In this paper is reported, based on the same synthetic scheme, the Dess-Martin periodinane oxidation of 9-HODE, 13-HODE, 9-HOTE and 13-HOTE either as free acids or as methyl ester derivatives, and the subsequent reduction of the formed keto compounds to yield back the hydroxy derivatives as racemic mixtures (see Scheme 1). In each case the variation of the stereochemistry of the conjugated dienic system was analysed by HPLC.

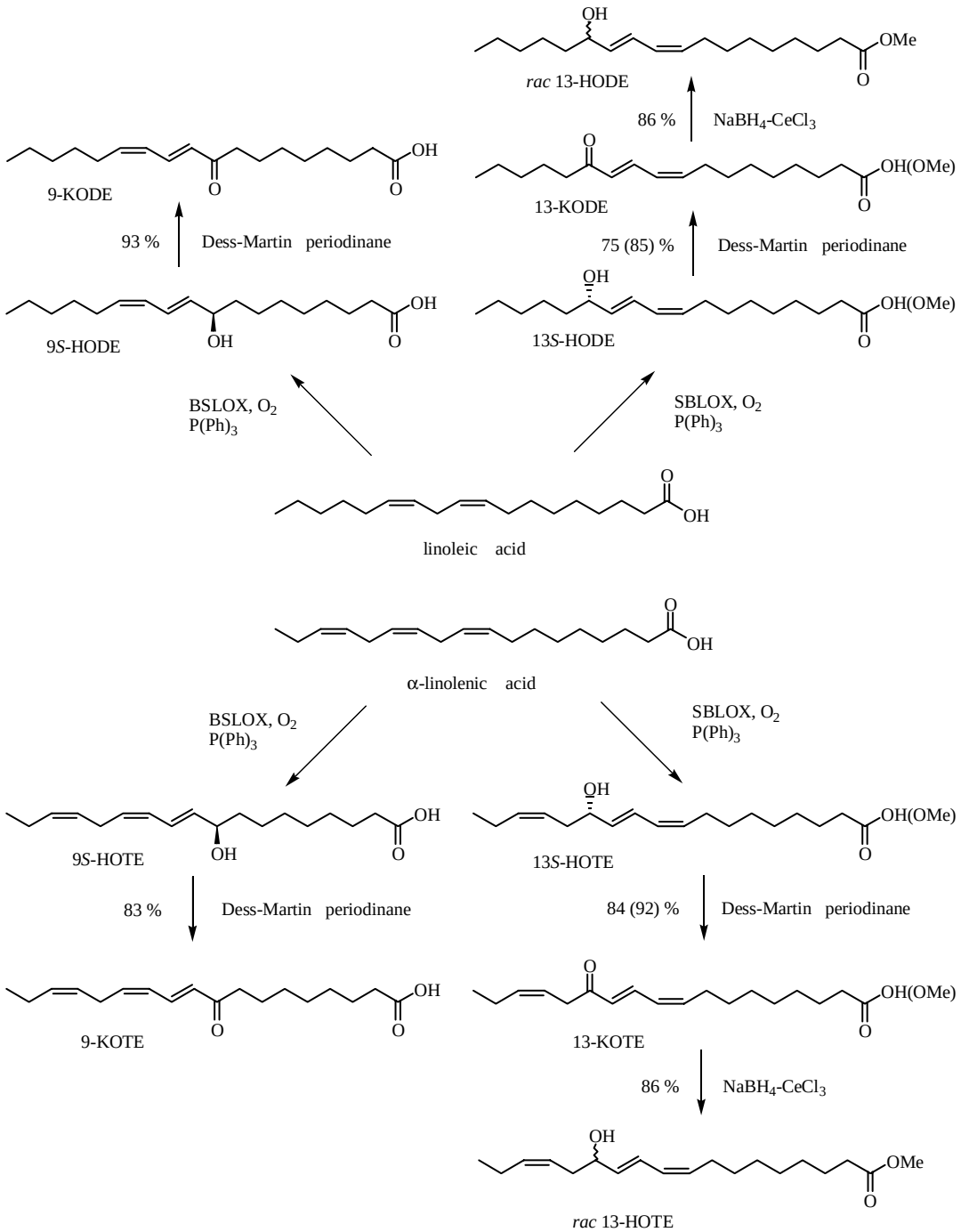
2. Experimental

2.1. General

Dichloromethane (Rectapur, Prolabo) was passed through activated silica and stored on molecular sieves prior to use. Methanol (RPE grade, Carlo Erba) was used without purification. Dess-Martin periodinane was purchased from Acros Organics as a 15 wt.% solution in dichloromethane and used as received, $CeCl_3{\cdot}7H_2O$ was from Lancaster and $NaBH_4$ (98%) from Aldrich. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were run on a Bruker AC 300 spectrometer in $CDCl_3$ with TMS as internal standard. Chemical shifts are given in ppm (δ) downfield relative to TMS. IR spectra were run on a Bruker IFS 25 spectrophotometer. UV spectra were recorded on a Uvikon 943 double beam UV-Vis spectrophotometer. Melting points were recorded on an Electrothermal 9300 apparatus and were uncorrected. Flash chromatography was performed on silica gel (Merck, Kieselgel 60; 230–400 mesh). The stereoisomeric distribution of either alcohol or keto fatty acids or methyl esters were determined by HPLC (Kontron HPLC pump 420) using a Waters Novapack Silica (2 mm × 150 mm) column. The UV detector (Kontron HPLC 335) was set at 234 nm for alcohols and 278 nm for keto derivatives. The used eluents were 100/1/0.1 for alcohols and 100/0.4/0.2 for keto derivatives (hexane/isopropanol/acetic acid) delivered, respectively at 0.7 and 0.8 ml/min.

2.2. General procedure for Dess-Martin periodinane oxidation

In a 100 ml dry round bottom flask equipped with a magnetic stirrer were weighted 1 mmol of hydroxy fatty acids. Then 40 ml of dichloromethane were added and the stirred mixture was cooled to 0 °C. Then 5 ml of Dess-Martin periodinane solution (1.7 equivalent) were added. After 5 min at 0 °C the reaction was completed (TLC analysis, solvent 70/30/0.2:



Scheme 1. Chemo-enzymatic transformation of linoleic and α-linolenic acids.

pentane/diethyl ether/acetic acid). The reaction mixture was then passed through a short pad of celite and silica gel and eluted with a mixture of 70/30/0.2: pentane/diethyl ether/acetic acid. After solvent evaporation the residue was purified using flash column chromatography over silica (solvent 50/50/0.2: pentane/diethyl ether/acetic acid). Fractions containing the keto derivative were then pooled and evaporated under vacuum. For methyl ester derivatives oxidation, the same procedure was adopted, excepted that acetic acid was omitted in solvent mixtures.

2.2.1. (10E,12Z)-9-Oxooctadecadienoic acid (9-KODE)

Yield: 93%, cream amorphous solid, mp 49.5–51.5 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J = 6.9 Hz, 3H), 1.33 (br s, 10H), 1.43 (m, 2H), 1.63 (m, 4H), 2.31 (t, J = 7.8 Hz, 2H), 2.34 (t, J = 7.3 Hz, 2H), 2.55 (t, J = 7.5 Hz, 2H), 5.91 (m, 1H), 6.12 (t, J = 11.2 Hz, 1H), 6.17 (d, J = 15.3 Hz, 1H), 7.50 (ddd, J = 15.3, 11.2, 0.6 Hz, 1H), 10.09 (br s, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 22.5, 24.3, 24.6, 28.3, 28.9, 29.0, 29.1, 29.1, 31.4, 34.0, 41.0, 126.9, 129.3, 137.2, 142.8, 180.0, 201.2, IR (KBr, neat): 3450, 2925, 2853, 1694, 1625, 1593, 1470, 1410, 1311, 1255, 1230, 1118, 996, 717 cm^{-1}, UV (EtOH): λ_{max} = 277.9 nm.

2.2.2. (10E,12Z,15Z)-9-Oxooctadecatrienoic acid (9-KOTE)

Yield: 83%, pale yellow amorphous sticky solid, mp 42.5–43.5 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J = 7.6 Hz, 3H), 1.34 (br s, 6H), 1.64 (m, 4H), 2.10 (m, 2H), 2.3 (t, J = 7.35 Hz, 2H), 2.56 (t, J = 7.4 Hz, 2H), 3.07 (t, J = 7.5 Hz, 2H), 5.25–5.55 (m, 2H), 5.86 (m, 1H), 6.14 (t, J = 11.3 Hz, 1H), 6.19 (d, J = 15.3 Hz, 1H), 7.53 (dd, J = 15.3, 11.3 Hz, 1H), 9.48 (br s, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.2, 22.7, 24.3, 24.6, 26.6, 28.9, 29.1, 29.1, 34.0, 41.0, 125.3, 126.9, 129.7, 133.3, 136.8, 140.2, 180.0, 201.1, IR (KBr, neat): 3453, 2927, 2853, 1697, 1621, 1591, 1470, 1409, 1309, 1247, 1229, 1116, 1072, 996, 720 cm^{-1}, UV (EtOH): λ_{max} = 277.3 nm.

2.2.3. (9Z,11E)-13-Oxooctadecatrienoic acid (13-KODE)

Yield: 75%, white amorphous sticky solid, mp 39.5–41.5 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (t, J = 6.8 Hz, 3H), 1.25–1.45 (m, 12H), 1.63 (m, 4H), 2.31 (m, 4H), 2.55 (t, J = 7.4 Hz, 2H), 5.90 (dt, J = 11.1, 7.8 Hz, 1H), 6.12 (t, J = 11.1 Hz, 1H), 6.18 (d, J = 15.3 Hz, 1H), 7.49 (ddd, J = 15.3, 11.1, 0.6 Hz, 1H), 10.09 (br s, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 22.4, 24.1, 24.6, 28.3, 28.9, 28.9, 29.0, 29.2, 31.5, 34.0, 41.2, 127.0, 129.4, 137.1, 142.5, 179.6, 201.4, IR (KBr, neat): 3450, 2925, 2850, 1694, 1618, 1588, 1468, 1409, 1265, 1220, 1192, 1077, 1000, 682 cm^{-1}, UV (EtOH): λ_{max} = 277.6 nm.

2.2.4. (9Z,11E,15Z)-13-Oxooctadecatrienoic acid (13-KOTE)

Yield: 84%, yellow viscous oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (t, J = 7.6 Hz, 3H), 1.25–1.50 (m, 8H), 1.63 (m, 2H), 2.00–2.15 (m, 2H), 2.25–2.40 (m, 4H), 3.32 (d, J = 6.6 Hz, 2H), 5.58 (m, 2H), 5.92 (dt, J = 11.1, 7.8 Hz), 6.12 (t, J = 11.1 Hz, 1H), 6.20 (d, J = 15.3 Hz, 1H), 7.53 (ddd, J = 15.3, 11.1, 0.6 Hz, 1H), 9.34 (br s, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 20.9, 24.6, 28.3, 28.9, 29.0, 29.0, 29.2, 34.0, 41.1, 120.6, 126.9, 128.7, 135.3, 137.7, 142.9, 179.8, 198.8, IR (thin film): 2931, 2853, 1709, 1628, 1587, 1461, 1412, 1276, 1189, 1091, 1069, 997, 725 cm^{-1}, UV (EtOH): λ_{max} = 279.9 nm.

2.2.5. (9Z,11E)-13-Oxooctadecatrienoic acid methyl ester (Me 13-KODE)

Yield: 85%, pale yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (t, J = 6.9 Hz, 3H), 1.25–1.50 (m, 12H), 1.55–1.70 (m, 4H), 2.25–2.35 (m, 4H), 2.55 (t, J = 7.3 Hz, 2H), 3.66 (s, 3H), 5.89 (dt, J = 11.1, 7.8 Hz, 1H), 6.12 (t, J = 11.1 Hz, 1H), 6.17 (d, J = 15.3 Hz, 1H), 7.49 (ddd, J = 15.3, 11.1, 0.9 Hz, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 22.5, 24.1, 24.9, 28.3, 29.0, 29.0, 29.1, 29.3, 31.5, 34.1, 41.1, 51.5, 127.0, 129.4, 136.9, 142.4, 174.3, 201.1, IR (thin film): 2930, 2857, 1740, 1688, 1666, 1630, 1591, 1462, 1437, 1412, 1250, 1220, 1195, 1175, 1084, 1056, 997, 871, 727 cm^{-1}, UV (EtOH): λ_{max} = 277.3 nm.

2.2.6. (9Z,11E,15Z)-13-Oxooctadecatrienoic acid methyl ester (Me 13-KOTE)

Yield: 92%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (t, J = 7.5 Hz, 3H), 1.315 (br s, 6H), 1.36–1.47 (m, 2H), 1.62 (m, 2H), 2.09 (m, 2H), 2.25–2.36 (m, 4H), 3.31 (d, J = 6.3 Hz, 2H), 3.66 (br s, 3H), 5.58 (m, 2H), 5.91 (dt, J = 11.4, 7.8 Hz),

6.12 (t, $J = 11.4$ Hz, 1H), 6.19 (d, $J = 15.3$ Hz, 1H), 7.53 (ddd, $J = 15.3$, 11.4, 0.9 Hz, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 20.9, 24.9, 28.4, 29.0, 29.0, 29.1, 29.3, 34.0, 40.1, 120.6, 126.9, 128.7, 135.2, 137.5, 142.8, 174.2, 198.5, IR (thin film): 2932, 2856, 1738, 1688, 1665, 1624, 1590, 1461, 1437, 1412, 1266, 1196, 1174, 1090, 1069, 997, 851, 730 cm^{-1}, UV (EtOH): $\lambda_{max} = 279.6$ nm.

2.3. General procedure for $NaBH_4$–$CeCl_3$ mediated reduction

In a 100 ml round bottom flask equipped with a magnetic stirrer were weighted 0.9 mmol of keto dienoic fatty acid methyl esters. Then 20 ml of methanol was added and the stirred mixture was cooled to 0 °C. Then 1 mmol of $CeCl_3 \cdot 7H_2O$ (1.1 equivalent) and $NaBH_4$ (1.1 equivalent) were added. After 5 min at 0 °C the reaction was completed (TLC analysis, solvent 60/40: pentane/diethyl ether). The reaction mixture was then passed through a short pad of celite rinsed with methanol. After solvent evaporation the oily residue was purified using flash column chromatography over silica (solvent 60/40: pentane/diethyl ether). Fractions containing the hydroxy derivatives were then pooled and evaporated under vacuum.

2.3.1. 13-hydroxy-9Z,11E-octadecadienoic acid methyl ester (Me 13-HODE)

Yield: 86%, colourless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, $J = 6.6$ Hz, 3H), 1.25–1.70 (br m, 20H), 2.18 (dt, $J = 6.9$, 6.9 Hz, 2H), 2.30 (t, $J = 7.5$ Hz, 2H), 3.66 (s, 3H), 4.10–4.20 (m, 1H), 5.43 (dt, $J = 10.9$, 7.8 Hz, 1H), 5.66 (dd, $J = 15.3$, 6.9 Hz, 1H), 5.97 (t, $J = 10.9$ Hz, 1H), 7.49 (dd, $J = 15.3$, 10.9, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.1, 22.6, 24.9, 25.1, 27.7, 29.0, 29.1, 29.1, 29.5, 31.8, 34.1, 37.3, 51.5, 72.9, 125.7, 127.8, 132.8, 136.0, 174.4, IR (thin film): 3426, 3005, 2920, 2856, 1738, 1462, 1436, 1362, 1247, 1198, 1084, 1019, 985, 950, 854, 726 cm^{-1}, UV (EtOH): $\lambda_{max} = 231.9$ nm.

2.3.2. 13-hydroxy-9Z,11E,15Z-octadecatrienoic acid methyl ester (Me 13-HOTE)

Yield: 86%, pale yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (t, $J = 7.5$ Hz, 3H), 1.2–1.5 (m, 8H), 1.62 (m, 2H), 2.0–2.4 (m, 8H), 3.66 (s, 3H), 4.21 (dt, $J = 6.6$, 6.6 Hz, 1H), 5.3–5.6 (m, 3H), 5.69 (dd, $J = 15.3$, 6.6 Hz, 1H), 5.97 (t, $J = 11.0$ Hz, 1H), 6.51 (dd, $J = 15.3$, 11.0, 1H), ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.2, 20.8, 27.7, 29.0, 29.1, 29.1, 29.5, 34.1, 35.3, 51.5, 72.1, 123.8, 123.8, 125.8, 127.8, 132.8, 135.1, 174.3, IR (thin film): 3433, 3007, 2931, 2855, 1741, 1462, 1436, 1366, 1247, 1199, 1174, 1091, 1038, 985, 869, 732 cm^{-1}, UV (EtOH): $\lambda_{max} = 233.9$ nm.

3. Results and discussion

3.1. Oxidation with Dess-Martin periodinane

The following compounds, 13-HODE, 13-HOTE, 9-HODE, 9-HOTE, Me 13-HODE and Me 13-HOTE were synthesised according to previously described procedures with SBLOX for 13-hydroperoxides and BSLOX for 9-hydroperoxides, followed by triphenylphosphine reduction (Iacazio et al., 1990; Martini et al., 1994; Martini et al., 1996; Martini and Iacazio, 1997). For each compounds the oxidation was conducted on the mmole scale (~300 mg) with 1.7 equivalent of Dess-Martin periodinane in CH_2Cl_2

Table 1
Evolution of the stereoisomeric distribution of conjugated dienes after Dess-Martin periodinane oxidation and $NaBH_4$–$CeCl_3$ reduction

Stereochemistry of the starting material (*ZE*/*EE*)	Stereochemistry of the products obtained through DMPO[a] (*ZE*/*EE*)	Stereochemistry of the products obtained through $NaBH_4$ reduction (*ZE*/*EE*)
13-HODE (98/2)	13-KODE (94/6)	
13-HOTE (98/2)	13-KOTE (98/2)	
9-HODE (97/3)	9-KODE (94/6)	
9-HOTE (98/2)	9-KOTE (96/4)	
Me 13-HODE (98/2)	Me 13-KODE (98/2)	*rac* Me 13-HODE (98/2)
Me 13-HOTE (97/3)	Me 13-KOTE (97/3)	*rac* Me 13-HOTE (97/3)

[a] DMPO: Dess-Martin periodinane oxidation.

at 0 °C. The reaction was shown to be extremely rapid (less than 5 min) and very clean (one spot to one spot by TLC). Purification over silica by flash chromatography afforded highly pure ketodienes in very good yield (75–93%) and with very little isomerisation as can be judged by HPLC (see Table 1). Structural determination was conducted by ^{1}H NMR, ^{13}C NMR, IR and UV spectroscopy and by comparison with literature data (Koch et al., 2002; Kuklev et al., 1997).

The reaction proved to be versatile since applicable to free fatty acids or their methyl esters, to doubly or triply unsaturated fatty acids and whatever the position of the hydroxy group. Thus, the combination of the well established chemoenzymatic synthesis of hydroxy derivatives of PUFA's with lipoxygenases and the chemical oxidation of such compounds using Dess-Martin periodinane allowed the very easy synthesis of a variety of ketodienes, relevant to plant physiology, in high yields and on the multi-hundred milligram scale.

3.2. Chemoselective reduction of Me 13-KODE and Me 13-KOTE

With these results in hand, it was therefore of interest also to study the reduction of the keto acids back to the hydroxy parent acids as racemic mixtures. Indeed almost all lipoxygenases are very enantioselective catalysts and this is a case where the enantiomerically nearly pure hydroxy acids are much more easily accessible than their racemic counterparts. The reducing system used was a $NaBH_4$–$CeCl_3$ mixture (Gemal and Luche, 1981) developed for the chemoselective reduction of enones. The reaction was conducted in MeOH at 0 °C allowing the synthesis of racemic methyl 13-HODE and methyl 13-HOTE in good yields (86%). Once again this transformation did not changed noticeably the stereochemistry of the conjugated dienic system as can be judged by HPLC (see Table 1). These compounds are valuable as standards for chiral HPLC and could be of interest in physiological studies.

4. Conclusion

In conclusion, a very efficient chemoenzymatic synthesis of various keto derivatives of fatty acids have been reported, by combining the use of lipoxygenases and Dess-Martin periodinane. The formed keto acids have almost retained the stereochemistry of their parent hydroxy compounds during the transformation. This reaction is insensitive to the number of unsaturation and to the position of the alcohol functionality and afforded, for the first time, multi-hundred milligrams of these highly valuable natural compounds. This transformation could also be coupled to the reduction with $CeCl_3$–$NaBH_4$ for the synthesis of racemic hydroxy fatty acids of fixed stereochemistry. Such racemic compounds are valuable as standards in chiral HPLC.

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