ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 6 (2): 283-287, 2007
ISSN 1682-296X

Biotransformation and Stereoselective Synthesis of Pharmaceutical Molecules from Linoleic Acid

[1]Muhammad Nor bin Omar, [1]Nor Nazuha M. Nor, [2]Humphrey Moynihan and [3]Richard Hamilton
[1]Malaysian Palm Oil Board (MPOB), No 6, Persiaran Institusi Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia
[2]Department of Chemistry, University College Cork, Cork, Ireland
[3]School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, L3 AF, UK

Abstract: The biotransformation of linoleic acid 1 using immobilised soybean lipoxygenase in a dimethyl sulfoxide (DMSO) containing medium followed by sodium borohydride reduction afforded 13(S)-hydroxy-octadeca-9(Z),11(E)-dienoic acid 13(S)-HODE 2 in 69% yield. After methylation, methyl 13(*S*)-HODE was subjected to epoxidation using tert-butyl hydroperoxide (TBHP) in the presence of vanadyl acetylacetonate or titanium (IV) isopropoxide with D-(-)- or L-(+)-diisopropyl tartrate. Epoxidation catalysed by titanium (IV) isopropoxide/D-(-)-isopropyl tartarate gave predominantly epoxide 3 (in 75% yield) while treatment of 2 with TBHP in the presence of titanium (IV) isopropoxide and L-(+)-diisopropyl tartrate gave preferentially epoxide 4 in 73% yield. Meanwhile, epoxidation of 2 with TBHP in the presence of vanadyl acetylacetonate gave predominantly epoxide 3 in 72% yield. The fluoro-derivatives 8, 9 and 10 were obtained in high yield when hydroxy-derivative 2, 3 and 4 were converted into their trimethylsilyl derivatives 5, 6 and 7 before treating with diethylaminosulphur trifluoride. Treatment of epoxide 4 with acetamide in dimethyl formamide (DMF) afforded a heterocyclic 2-oxazoline 11 in 73% yield.

Key words: Biotransformation, linoleic acid, lipoxygenase, pharmaceutical molecules

INTRODUCTION

Oil and fat products, particularly fatty acids and their metabolites, play important roles in plants and mammals as defensive agents against pathogenic attacks and diseases (Schaller, 2001). The metabolites are of increasing interest in a variety of applications, for example, as anti-fungal substance, precursor in the formation of cutin (Kallio *et al*., 2006; Blee, 2002) and intermediates in the formation of jasmonic acid, the plant hormone (Schaller, 2001). In mammals, bioactive metabolites display unique calcium ionophoric properties, decreases platelet adhesion to endothelial cell and maintains the thromboresistance and chemorepellant of vascular endothelium (Prakesch *et al*., 2005, Verma *et al*., 2003, Lee *et al*., 2001). However, most vegetable oils and fatty acids are used in food industries such as in the production of margarine, shortening, fat spread, ice cream, bakery and frying medium for fried and snack food products. As a result, the utilization of fatty acids as synthetic starting materials in pharmaceutical applications have not been explored widely. Previous studies showed that enzymatic biotransformation of unsaturated fatty acids, particularly linoleic acid, into chiral hydroxyperoxides (Márczy *et al*., 2002; Matsui *et al*., 2003) would increase the synthetic utility of these materials. Thus, the present study aims to transform fatty acid, especially linoleic acid, into value-added chiral metabolites, i.e., hydroxy acids, epoxides, fluoro derivatives and heterocyclic compound, which exhibit biological and pharmaceutical properties.

MATERIALS AND METHODS

General procedures: Enzymatic and chemical syntheses were carried out at Chemistry Department, Liverpool John Moores University, United Kingdom. Optical rotations were recorded on a Model D Polarimeter (Bellingham and Stanley, UK). Infrared spectra (ν_{max}) were recorded on a Perkin-Elmer 1600 series FTIR spectrometer. ^{1}H NMR spectra were recorded on a JOEL JNM-FX90Q FT-NMR spectrometer; samples were dissolved in $CDCl_3$ or d_6 DMSO and chemical shifts (δ_H) are reported in ppm downfield of tetramethylsilane. Coupling constants (J)

Corresponding Author: Muhammad Nor bin Omar, Malaysian Palm Oil Board (MPOB), No 6, Persiaran Institusi Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia
Tel: +603-8769 4439 Fax +603-89259446

are quoted in Hz. Mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service Centre, University of Swansea, UK. Silica gel column chromatography was performed using silica gel G60, 70-230 mesh (Merck Ltd). Methyl esters were prepared by treatment of the carboxylic acids with ethereal solutions of diazomethane. Hydroxy-compounds were converted into trimethylsilyl ethers prior to analysis by treatment with N,O-bis(trimethylsilyl)trifluoroacetamide at 60°C for 15 min.

Chemoenzymatic conversion of linoleic acid 1: The soybean lipoxygenase type 1B was immobilised on oxirane acrylic beads as described earlier (Maguire *et al*., 1991). The incubation of linoleic acid in dimethyl sulfoxide (DMSO) was carried out according to previously reported method (Omar *et al*., 2003a). After incubation, the suspension was acidified to pH 3 and extracted with diethyl ether. The combined ethereal layer was dried and concentrated under reduced pressure. The residue was dissolved in methanol, cooled at 0°C and reduced with sodium borohydride. Purification using silica gel column chromatography with 30% diethyl ether in hexane containing 1% glacial acetic acid afforded 13S-HODE (2) as a colourless oil (69%, $[\alpha]_D^{25}$ + 9.6° (c = 0.6 $CHCl_3$); ν_{max}/cm^{-1} (film) 3443, 2997, 2920, 2845, 1707, 896; δ_H 0.91 (3H, t, CH_3), 1.26 - 1.68 (18H, m, CH_2), 2.20 (2H, m, 8-CH_2), 2.36 (2H, t, 2-CH_2), 4.10 (1H, m, H-13), 5.41 (1H, t, J = 10.7, H-9), 5.70 (1H, m, H-12), 5.80 (2H, s, OH and CO_2H), 5.93 (1H, d, H-10), 6.43 (1H, m, J = 15.3, H-11); m/z (Trimethylsilylated methyl ester) (EI) 73 (100%), 74, 173, 225, 293, 311, 367, 382 (M^+).

Preparation of epoxides: The epoxidation of 13S-HODE 2 was performed according to reported procedure (Omar *et al*., 2003b). The epoxides formed were extracted with dichloromethane, washed with aqueous ferrous sulphate, dried and concentrated *in vacuo*. The residue was subjected to silica gel chromatography using diethyl ether/hexane (30/70) containing 1% triethylamine as eluant. Epoxidation of methyl 13(S)-HODE 2 in the presence of D-(-)-diisopropyltartrate gave 3 (78 mg, 75%) and 4 (11 mg, 11%). Epoxidation of methyl 13(S)-HODE 2 in the presence of L-(+)-diisopropyltartrate gave 3 (6%) and 4 (73%), respectively.

Meanwhile, vanadyl-catalysed epoxidation of 2 followed the method of Falck *et al*. (1983). The epoxides formed were extracted with dichloromethane, washed with aqueous ferrous sulphate, dried and concentrated under reduced pressure. Purification using silica gel chromatography with 30% diethyl ether in hexane containing triethylamine gave 3 (72%) and 4 (6%), respectively.

Methyl 11(S), 12(R)-epoxy-13(S)-hydroxy-9(Z)-octadecenoate (3): $[\alpha]_D^{25}$ + 2.1° (c = 1.0 $CHCl_3$); ν_{max}/cm^{-1} (film) 3445, 2997, 2921, 2856, 1743, 849; δ_H 0.91 (3H, t, CH_3), 1.21 - 1.62 (18H, m, CH_2), 2.23 - 2.38 (4H, m, H-2, H-8), 2.93 (1H, dd, $J_{11\text{-}12}$ = 2, $J_{12\text{-}13}$ = 5, H-12), 3.62 (1H, m, H-13), 3.74 (3H, s, OCH_3), 3.81 (1H, dd, $J_{10\text{-}11}$ = 8, $J_{11\text{-}12}$ = 2, H-11), 5.39 (1H, dd, $J_{9\text{-}10}$ = 11, $J_{10\text{-}11}$ = 8, H-10), 5.82 (1H, dt, $J_{8\text{-}9}$ = 7, $J_{9\text{-}10}$ = 11, H-9); m/z (trimethylsilyl ether) (EI) 41, 55, 67, 73, 74, 75, 81, 95, 155, 173, 187, 199, 270, 327, 367, 383. [M+H] 399.3088 (calc); observed (CI) 399.3086.

Methyl 11(*R*), 12(*S*)-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate (4): $[\alpha]_D^{25}$ + 4.5° (c = 1.0 $CHCl_3$); ν_{max}/cm^{-1} (film) 3468, 2997, 2930, 2857, 1741, 893; δ_H 0.91 (3H, t, CH_3), 1.21-1.62 (18H, m, CH_2), 2.23 - 2.38 (4H, m, H-2, H-8), 2.90 (1H, dd, $J_{11\text{-}12}$ = 2, $J_{12\text{-}13}$ = 3, H-12), 3.74 (3H, s, OCH_3), 3.79 (1H, dd, $J_{10\text{-}11}$ = 6, $J_{11\text{-}12}$ = 2, H-11), 4.01 (1H, m, H-13), 5.30 (1H, dd, $J_{9\text{-}10}$ = 11, $J_{10\text{-}11}$ = 9, H-10), 5.86 (1H, dt, $J_{8\text{-}9}$ = 8, $J_{9\text{-}10}$ = 11, H-9); m/z (trimethylsilyl ether) (EI) 41, 55, 67, 73, 74, 75, 81, 95, 155, 173, 187, 199, 270, 327, 367, 383. [M+H] 399.3088 (calc); observed (CI) 399.3086.

Preparation of fluorinated derivatives

Fluorination with diethylaminosulphur trifluoride (DAST): Substrate (100 mg) was dissolved in N,O-bis (trimethylsilyl) trifluoroacetamide (0.5 mL) and heated at 90°C for 30 min, after which the excess N,O-bis (trimethylsilyl)- trifluoroacetamide was evaporated *in vacuo*. The residue was dissolved in dichloromethane (10 mL) and cooled to -78°C. A solution of diethylaminosulphur trifluoride (0.1 mL, 0.5 mmol) in dichloromethane (1 mL) was added and the reaction mixture was stirred at -78°C for 1 h and at ambient temperature for 30 min, after which water (100 mL) was added and the mixture washed with aqueous sodium bicarbonate (0.1 M, 50 mL), dried over magnesium sulphate, filtered and the solvent evaporated under a stream of nitrogen. The residue was subjected to silica gel chromatography using diethyl ether/hexane (15/85) containing 1% triethylamine as eluant.

Methyl 13(R)-fluorooctadeca-9(Z), 11(E)-dienoa-te (8): Methyl 13(S)-HODE 2 (0.32 mmol) gave 8 in 86% yield; $[\alpha]_D^{25}$ + 4.5° (c = 1.0 $CHCl_3$); ν_{max}/cm^{-1} (film) 2997, 1735, 984, 783; δ_H 0.91 (3H, t, CH_3), 1.29 - 1.42 (16H, m, CH_2), 1.84 (2H, m, H-14), 2.17 (2H, m, H-8), 2.30 (2H, t, *J* = 7, H-2), 3.65 (3H, s, OCH_3), 4.82 (1H, m, J_{HF} = 48, $J_{H12,H13}$ = 7, H-13), 5.54 (1H, m, $J_{H9,H10}$ = 10, H-9), 5.75 (1H, dd, $J_{H11,H12}$ = 15, $J_{H12,H13}$ = 7, H-12), 6.05 (1H, dd, $J_{H10,H11}$ = 11, $J_{H9,H10}$ = 10, H-10), 6.50 (1H, dd, $J_{H11,H12}$ = 15, $J_{H10,H11}$ = 11, H-11); m/z (EI) 55 (100%), 74, 218, 260, 292, 293, 312 (M^+).

Methyl 11(*S*), 12(*R*)-epoxy-13(*R*)-fluoro-9(*Z*)-octadecenoate (9): Methyl 11(S),12(R)-epoxy-13(S)-hydroxy-9(Z)-octadecenoate 3 (0.32 mmol) gave 9 in 87% yield. $[\alpha]_D^{25}$ - 13.5° (c = 0.8 $CHCl_3$); ν_{max}/cm^{-1} (film) 3004, 2921, 2856, 1740, 849, 784; δ_H 0.91 (3H, t, CH_3), 1.21-1.62 (20H, m, CH_2), 2.38 (2H, t, H-2), 2.96 (1H, dd, $J_{H12,H13}$ = 9, $J_{H11,H12}$ = 2, H-12), 3.74 (3H, s, OCH_3), 3.79 (1H, dd, $J_{H10,H11}$ = 6, $J_{H11,H12}$ = 2, H-11), 4.76 (1H, m, J_{HF} = 47, J = 2, H-13), 5.30 (1H, dd, $J_{H9,H10}$ = 11, $J_{H10,H11}$ = 6, H-10), 5.63 (1H, dt, $J_{H9,H10}$ = 11, $J_{H8,H9}$ = 8, H-9); m/z (EI) 41, 55 (100%), 67, 73, 74, 75, 81, 95, 135, 155, 173, 187, 199, 225, 251, 277, 291, 308, 309, 328 (M^+). [M + NH_4] 346.2901 (calc); observed (CI) 3346.2905.

Methyl 11(R),12(S)-epoxy-13(R)-fluoro-9(Z)-octadecenoate (10): Methyl 11(R),12(S)-epoxy-13(S)-hydroxy-9(Z)-octadecenoate 4 (0.31 mmol) gave 10 in 89% yield, $[\alpha]_D^{25}$ -9.8° (c = 0.8 $CHCl_3$); ν_{max}/cm^{-1} (film) 3005, 2931, 2858, 1740, 843, 789; δ_H 0.91 (3H, t, CH_3), 1.21-1.62 (20H, m, CH_2), 2.38 (2H, t, H-2), 2.97 (1H, dd, J = 2, J = 2, H-12), 3.74 (3H, s, OCH_3), 3.79 (1H, dd, J = 6, J = 2, H-11), 4.78 (1H, dt, J_{HF} = 49, J = 2, H-13), 5.30 (1H, dd, J = 11, J = 8, H-10), 5.65 (1H, dt, J = 11, J = 8, H-9); m/z (EI) 41, 55 (100%), 67, 73, 74, 75, 81, 95, 135, 155, 173, 199, 225, 251, 277, 291, 308, 309, 328 (M^+). [M + NH_4] 346.2906 (calc); observed (CI) 346.2903.

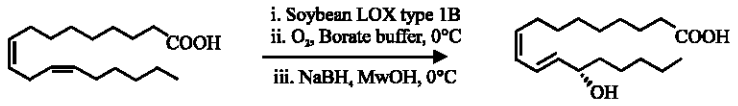
Preparation of 2-oxazoline: A solution of epoxide 4 (50 mg, 0.15 mmol) and acetamide (9 mg, 0.15 mmol) in DMF (20 mL) was heated to 100°C for 24 h, after which the solvent was removed *in vacuo* and the residue subjected to silica gel chromatography using diethyl ether/ hexane (10/90) as eluant.

4R-(9-carboxymethyl-1-nonenyl)-5R-(1R-hydroxyhexyl)-2-methyl-2-oxazoline (11): Methyl 11(R),12(S)-epoxy-13(S)-hydroxy-9(Z)-octadecenoate 4 gave oxazoline 11 in 73% yield, $[\alpha]_D^{25}$ + 7.2° (c = 1.0 $CHCl_3$); ν_{max}/cm^{-1} (film) 3454, 1735, 1655, 1372, 722; δ_H 0.91 (3H, t, CH_3), 1.25-1.90 (25H, m, CH_3, CH_2), 3.85 (1H, m, J = 9, CHOH), 3.62 (3H, s, OCH_3), 3.75 (1H, m, J = 9, H-5), 4.01 (1H, s, OH), 4.75 (1H, m, J = 9, H-4) 5.30 (2H, m, J = 11, J = 9, CH = CH); m/z 73 (100%), 74, 75, 173, 198, 368, 408, 421, 424, 440 $[M+H]^+$. $[M+H]^+$ 440.2356 (calc); observed (CI) 440.2353.

RESULTS AND DISCUSSION

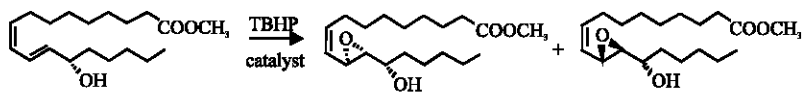
Biotransformation of linoleic acid 1 into 13S-HODE 2: The production of 13S-HODE 2 by oxidation of linoleic acid 1 (Scheme 1) using immobilised soybean lipoxygenase (Maguire *et al*., 1991) in a DMSO-containing medium was carried out (Omar *et al*., 2003a). This procedure gave preparative quantities of 13(S)-HODE 2 in 69% yield and in excellent optical purity (Scheme 1). This yield was better than the resultant product 2 from linoleic acid 1 after oxidation with LOX without immobilization and the addition of DMSO (Omar *et al*., 2003a). The high yield and good optical purity of 13S-HODE could be used as an important chiral source for further transformation into the other bioactive metabolites.

Epoxide metabolites of Methyl 13S-HODE: Epoxidation of 2 using external epoxidising agent, i.e., tert-butyl hydroperoxide gave a better yield with good diastereoselectivity. Epoxidation occurred specifically at the 11(Z)-alkene closer to the hydroxyl in all cases (Scheme 2). Epoxidation using tert-butyl hydroperoxide (TBHP) catalysed by titanium (IV) isopropoxide in the presence of D-(-)-diisopropyl tartrate gave selectively methyl (11R, 12R)-trans-epoxi-13S-hydroxy-9Z-octadece noate 3 in 75% yield whilst treatment of 2 with TBHP in the presence of vanadyl acetylacetonate gave



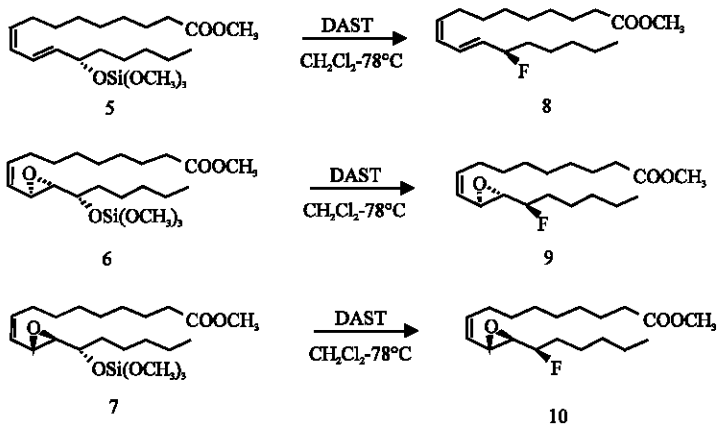
1 2

Scheme 1:

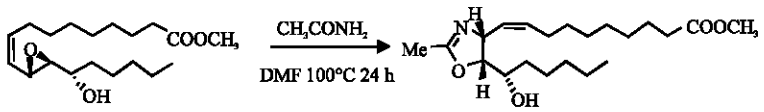


2 3 4

Scheme 2:



Scheme 3:



Scheme 4:

predominantly epoxide 3 in 72% yield. However, epoxidation of 2 with TBHP/ titanium (IV) isopropoxide in the presence of L-(+)-diisopropyl tartrate was found to give a reversal in diastereoselectivity, with methyl (11S,12S)-trans-epoxi-13S-hydroxy-9Z-octadecenoate 4 being obtained in higher quantity. The spectroscopic data of epoxides 3 and 4 (Scheme 2) were in agreement with previously reported data (Piazza *et al*., 1997; Omar *et al*., 2003b)

Fluoro-derivatives of 13S-HODE metabolites: Scheme 3 shows the trimethylsilyl- and fluoro-derivatives obtained upon treatment of the hydroxy- and epoxyhydroxy-fatty acid esters described above with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and diethylamino-sulphur trifluoride (DAST). Conversion of 2 into trimethylsilyl derivative 5 followed by treatment with DAST afforded methyl 13(R)-fluorooctadeca-9(Z),11(E)-dienoate (8) in 86% yield. Meanwhile, epoxides 3 and 4 gave methyl 11(S),12(R)-epoxy-13(R)-fluoro-9(Z)-octadecenoate(9)and methyl 11(R),12(S)-epoxy-13(R)-fluoro-9(Z)-octoctadece-noate (10) in 87 and 89% yield, respectively. It was found that much improved yields were obtained by conversion of the substrates into trimethylsilyl ethers by treatment with BSTFA prior to reaction with DAST. This is because the trimethylsilyl ether group could serve as a good leaving group for the fluorine atom replacement and the reaction was found to proceed with inversion of stereochemistry in each case (Matsumura *et al*., 1995).

Heterocyclic compound from epoxide derivative: The investigation on the conversion of epoxides 4 into 2-oxazoline derivative 11 by reaction with acetamide in DMF at 100°C (Ansari and Ahmad, 1987). One isomer was only isolated and via the coupling constant indicated that this derivative had cis-stereochemistry at positions 4 and 5 of the oxazoline ring. This stereochemistry is in agreement with previous reported finding which indicated that the reaction of trans epoxide with acetamide afforded the 2-oxazoline in *cis* configuration (Wohl and Cannie, 1973). It is therefore believed that this to be the oxazoline 11 (Scheme 4).

CONCLUSIONS

Biotransformation of an achiral linoleic acid 1 followed by chemical reduction afforded an optically active metabolite 13S-HODE 2 in good yield with excellent stereochemistry. 13S-HODE 2 was then used as a chiral starting material for a wide range of molecules such as epoxides, fluorinated derivative and heterocyclic 2-oxazoline. Epoxy compounds 3 and 4 were synthesized by the treatment of 13S-HODE 2 with *tert*-butyl hydroperoxide (TBHP) in the presence of vanadyl acetylacetonate or titanium (IV) isopropoxide/dialkyl tartarate. The epoxide 4 was then converted into 2-oxazoline 11 by treating with acetamide in DMF. The fluoro-derivatives 8, 9 and 10 were obtained in high yield when hydroxy-derivative 2, 3 and 4 were converted into their trimethylsilyl derivatives 5, 6 and 7 before treating with diethylaminosulphur trifluoride (DAST).

ACKNOWLEDGMENTS

The authors wish to thank the Director General of the Malaysian Palm Oil Board (MPOB) for financial support and Liverpool John Moores University for permission to use synthesis laboratory. Also thank to the EPSRC National Mass Spectrometry Service Centre, Swansea, UK for analytical assistance.

REFERENCES

Ansari, M.H. and M. Ahmad, 1987. Synthesis of fatty 2-oxazoline based on fatty methyl 2, 3-epoxy ester. Chem. Ind., pp: 95-95.

Blee, E., 2002. Cutin Monomers Biosynthesis and Plant Defence. In: Lipid Biotechnology. Kuos, T.M. (Eds.), Marcel Deccer Inc., New York, pp: 231-248.

Falck, J.R., S. Manna, A.K. Siddhanta, J. Capdevila and J.D. Buynak, 1983. Transformation of 15-HETE to 14, 15-dihydroxyeicosatrienoic acid and 11, 14, 15- and 13, 14, 15-trihydroxyeicosatrienoic acids. Tetrahedron Lett. 24: 5715-5718.

Kallio, H., R. Nieminen, S. Tuomasjukka and M. Hakala, 2006. Cutin composition of five finnish berries. J. Agric. Food Chem., 54: 457-462.

Lee, Y.W., H. Kühn, S. Kaiser, B. Hennig, A. Daugherty and M. Toborek, 2001. Interleukin 4 induces transcription of the 15-lipoxygenase I gene in human endothelial cells. J. Lipid Res., 42: 783-791.

Maguire, N.M., M.F. Mahon, K.C. Molloy, G. Read, S.M. Roberts and V. Sik, 1991. Chemoenzymatic synthesis of some macrocyclic C13-lactones. J. Chem. Soc., Perkin Trans., 1: 2054-2056.

Márczy, J.S., Á.S. Németh, Z. Samu, Á.H. Veress and B. Szajáni, 2002. Production of hexanal from hydrolyzed sunflower oil by lipoxygenase and hydroperoxid lyase enzymes. Biotechnol. Lett., 24: 1673-1675.

Matsui, K., S. Sasahara, Y. Akakabe and T. Kajiwara, 2003. Linoleic acid 10-hydroperoxide as an intermediate during formation of 1-octen-3-ol from linoleic acid in *Lentinus decadetes*. Biosci. Biotech. Biochem., 67: 2282-2282.

Matsumura, Y., T. Shimada, T. Nakayama, M. Urushihara and T. Asai *et al*., 1995. Synthesis of 7-fluoro-2, 4-methylene-17, 20-dimethylprostacyclins. Novel stable prostacyclin analogs as potent anti-anginal agents. Tetrahedron, 51: 8771-8782.

Omar, M.N., H. Moynihan and R. Hamilton, 2003a. Scaling-up the production of 13S-hydroxy-9Z, 11E-octadecadienoic acid (13S-HODE) through chemoenzymatic technique. Bull. Korean Chem. Soc., 24: 397-399.

Omar, M.N., H.A. Moynihan and R.J. Hamilton, 2003b. Asymmetric sharpless epoxidation of 13S-hydroxy-9Z, 11E-octadecadienoic acid (13S-HODE). Eur. J. Lipid Sci. Technol., 105: 43-44.

Piazza, G.J., T.A. Foglia and A. Nunez, 1997. Enantioselective formation of an α, β-epoxy alcohol by reaction of methyl 13(S)-hydroperoxy-9(Z),11(E)-octadecadienoate with titanium isopropoxide. JAOCS., 74: 1385-1390.

Prakesch, M., D. Gree, S. Chandrasekhar and R. Gree, 2005. Synthesis of fluoro analogues of unsaturated fatty acids and corresponding acyclic metabolites. Eur. J. Org. Chem., 7: 1221-1232.

Schaller, F., 2001. Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. J. Exp. Bot., 52: 11-23.

Verma, S., M.R. Buchanan and T.J. Anderson, 2003. Endothelial function testing as a biomarker of vascular disease. Circulation, 108: 2054.

Wohl, R.A. and J. Cannie, 1973. The stereochemistry of 2-oxazoline formation from epoxides. J. Org. Chem., 38: 1787-1790.