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Enzyme-catalyzed Preparation of Novel Fatty Acid Derivatives of Pyridoxine with Surfactant Activity

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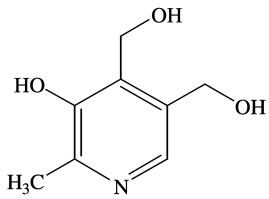
A series of novel fatty acid derivatives of pyridoxine, one of the three members of the vitamin B_6 group, has been prepared. These products were obtained using an enzymatic approach. Several lipases catalyzed esterification and transesterification reactions of pyridoxine with carboxylic acid or alkyl carboxylates showed a remarkable regioselective behavior; only monoacyl derivatives were obtained. The surfactant activity, composition and clean enzymatic methodology applied in the preparation of these products make them useful as ingredients in cosmetic and pharmaceutical formulations or food additives.

Keywords: Enzyme-catalyzed; Pyridoxine; Fatty acids; Surfactants

INTRODUCTION

In recent years, lipases have become attractive as biocatalysts in a number of reactions performed in organic media (Wong and Whitesides, 1994). The biocatalytic methodology is highly regio and stereo selective (Faber, 2000). It is also easy to carry out under mild conditions and is environmentally friendly. Within the scope of our general interest in applied biocatalysis, we recently reported (Baldessari *et al.*, 1998) a series of lipase-catalyzed acylation and deacylation reactions of pyridoxine **1**, one of three members of the vitamin B_6 group.

Vitamin B_6 can be used in cosmetic formulations (Snider and Dietman, 1974) and food additives (Driskell, 1994). Less polar derivatives of these compounds, such as esters that have higher affinity for membrane lipids and increased skin penetration could also be useful for these purposes. The ester groups can be easily hydrolyzed *in vivo* providing in this case two natural active molecules: vitamin and fatty acid. The novel compounds described here can be used as ingredients in cosmetic formulations such as sensitive skin shampoos and after sun exposure creams (Baldessari and Mangone, 2000).



1

In addition, because of the surfactant activity of the fatty acid derivatives of vitamin B_6 reported in this paper, they show good potential as food additives (Bowen, 1994) and pharmaceuticals (Wyatt, 1999). It is well known that compounds rich in high-value polyunsaturated fatty acids have beneficial therapeutic (Angerer and von Schacky, 2000) and nutritional effects (Simopoulos, 1999).

The three hydroxy groups of pyridoxine make the selective esterification at a specific position of the pyridoxine molecule difficult (Korytnyk and Paul, 1967). Attempts to acylate **1** under controlled conditions to produce monoacylated compounds always needed protection and deprotection reactions, otherwise, the result would be a mixture of esters in which the desired compound could be isolated only after careful separation (Brown *et al.*, 1993). As a result of our work on lipase-catalyzed

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regioselective acylation and deacylation reactions of pyridoxine, this paper reports on the preparation of novel fatty acid derivatives of pyridoxine by the enzymatic methodology.

MATERIALS AND METHODS

Materials

Pyridoxine, fatty acids, lipase from *Candida cylindracea* (905 units/mg solid) and lipase (type II crude) from porcine pancreas (190 units/mg protein) were purchased from Sigma Chemical Co. Lipozyme (lipase IM-60 from *Rizomucor miehei* in the immobilized form on a microporous anion exchange resin) and *Candida antarctica* lipase (Novozym 435 (7400 PLU/g) acrylic resin supported lipase produced by a host organism *Aspergillus oryzae*, after transfer of the gene encoding lipase B from *Candida antarctica*) were generous gifts of Novo Nordisk Bioindustrial Group. All enzymes were used "straight from the bottle".

Analytical

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Thin layer chromatography (TLC) was performed with Merck Silica gel 60F-254 aluminum sheets (0.2 mm thickness). For column chromatography Merck Silica gel 60 (0.040–0.063 mm) was used. ^{1}H-NMR and ^{13}C-NMR spectra were measured at 200 and 50 MHz, respectively, using a Bruker AC-200 spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) as internal standard, using $CDCl_3$ and CD_3OD as solvents. Assignments were based on COLOC and HETCOR spectra. EI-MS were obtained at 70 eV using a TRIO-2 VG Masslab and Shimadzu QP-5000 mass spectrometers, in m/z (%). High-resolution mass spectra were recorded on a ZAB BEqQ instrument. IR spectra were measured on a Nicolet-Magna-550-FT/IR spectrophotometer, in cm^{-1}.

Enzymatic Preparation

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Octadecanoate (2a)

To a solution of **1** (200 mg, 1.2 mmol) in acetone (10 ml) containing ethyl octadecanoate (850 mg, 3 mmol), 0.5 g of CAL were added. The suspension was stirred (200 rpm) for 72 h at 30°C and the progress of reaction was monitored by TLC. CH_2Cl_2 and MeOH (2:1) were added to the reaction mixture and the enzyme filtered off. The mixture was concentrated *in vacuo* and purified by silica gel chromatography (ethyl acetate/hexane (1:3)):355 mg (69%) of **2a**. M.p.: 96–97°C. IR (thin film, $CHCl_3$): 3444, 3045, 2924, 2859, 2703, 1733, 1483, 1241, 1177, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.91 (s, 1H, H-6), 5.02 (s, 2H, H-9), 4.99 (s, 2H, H-8), 2.45 (s, 3H, H-7), 2.28 (t, 2H, $J = 7.8$ Hz, H-11), 1.59 (m, 2H, H-12), 1.25 (m, 30H, H-13 a H-26), 0.88 (t, 3H, $J = 6.9$ Hz, H-27); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.53 (C-10), 151.46 (C-3), 147.92(C-2), 139.07 (C-6), 130.96 (C-4), 126.32 (C-5), 61.12 (C-9), 59.40 (C-8), 33.91 (C-11), 31.65 (C-12), 29.40, 29.18, 29.06, 28.95, 28.85 (C-13 to C-23), 24.74 (C-24), 24.61 (C-25), 22.39 (C-26), 17.79 (C-7), 13.71 (C-27). EI-MS m/z (relative intensity): 435 [M$^+$] (1), 151 (100), 123 (36), 106 (15), 85 (15), 73 (49), 57 (75), 43 (96).HR-MS: 435.3334 ($C_{26}H_{45}NO_4^+$; calc. 435.3349).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Eicosanoate (2b)

As described for **2a**, but using eicosanoic acid (940 mg, 3 mmol) as acylating agent and stirring the suspension for 96 h: 350 mg (64%). M.p.: 99–100°C. IR (thin film, $CHCl_3$): 3443, 2923, 2855, 1711, 1462, 1307, 1105, 1044 cm^{-1}; ^{1}H-NMR ($CDCl_3$:CD_3OD) δ (ppm): 7.89 (s, 1H, H-6), 5.06 (s, 2H, H-9), 4.95 (s, 2H, H-8), 2.46 (s, 3H, H-7), 2.34 (t, 2H, $J = 7.8$ Hz, H-11), 1.62 (m, 2H, H-12), 1.30 (m, 34H, H-13 a H-28), 0.90 (t, 3H, $J = 6.9$ Hz, H-29); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.97 (C-10), 151.92 (C-3), 148.31 (C-2), 139.47 (C-6), 131.36 (C-4), 126.73 (C-5), 61.53 (C-9), 59.78 (C-8), 34.31 (C-11), 32.05 (C-12), 29.81, 29.73, 29.60, 29.49, 29.35, 29.24 (C-13 to C-26), 25.01 (C-27), 22.80 (C-28), 18.20 (C-7), 14.12 (C-29). EI-MS: 463 [M$^+$](1), 151 (100), 123 (20), 106 (12), 83 (13), 73 (12), 57 (54), 43 (100). HR-MS: 463.3661 ($C_{28}H_{49}NO_4^+$; calc. 463.3662).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl **Cis-9-octadecenoate (2c)**

As described for **2a**, but using *cis*-9-octadecenoic acid (1.1 ml, 3 mmol) as acylating agent: 308 mg (60%). M.p.: 37–38°C. IR (thin film, $CHCl_3$): 3451, 3016, 2931, 2859, 2710, 1740, 1462, 1248, 1163, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.89 (s, 1H, H-6), 5.33 (m, 2H, H-18 and H-19), 5.03 (s, 2H, H-9), 4.99 (s, 2H, H-8), 2.44 (s, 3H, H-7), 2.28 (t, 2H, $J = 7.8$ Hz, H-11), 2.00 (m, 4H, H-17 and H-20), 1.59 (m, 2H, H-12), 1.27 (m, 20H, H-13 to H-16 and H-21 to H-26), 0.88 (t, 3H, $J = 6.9$ Hz, H-27); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.29 (C-10), 152.14 (C-3), 148.28 (C-2), 139.25 (C-6), 130.90 (C-4), 130.06, 129.69 (C-22 and C-23), 126.40 (C-5), 61.23 (C-9), 60.29 (C-8), 34.15 (C-11), 31.91 (C-12), 29.78, 29.70, 29.54, 29.32, 29.16, 27.25, 27.17 (C-13 to C-24), 24.88 (C-25), 22.69 (C-26), 18.19 (C-7), 14.12 (C-27). EI-MS: 433 [M$^+$](1), 151 (83), 123 (48), 106 (23), 83 (18), 69 (31), 57 (40), 55 (79), 43 (90), 41 (100). HR-MS: 433.3213 ($C_{26}H_{43}NO_4^+$; calc. 433.3192).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Trans*-9-octadecenoate (2d)*

As described for **2a**, but using *trans*-9-octadecenoic acid (850 mg, 3 mmol) as acylating agent: 261 mg (51%). M.p.: 47–48°C. IR (thin film, $CHCl_3$): 3437, 3038, 2916, 2852, 2703, 1740, 1469, 1248, 1177, 1035 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.88 (s, 1H, H-6), 5.37 (m, 2H, H-18 and H-19), 5.03 (s, 2H, H-9), 4.98 (s, 2H, H-8), 2.44 (s, 3H, H-7), 2.28 (t, 2H, J = 7.8 Hz, H-11), 1.96 (m, 4H, H-17 and H-20), 1.58 (m, 2H, H-12), 1.27 (m, 20H, H-13 to H-16 and H-21 to H-26), 0.87 (t, 3H, J = 6.9 Hz, H-27); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.21 (C-10), 152.19 (C-3), 148.09 (C-2), 138.87 (C-6), 130.98 (C-4), 130.41, 130.03 (C-18 and C-19), 126.34 (C-5), 61.09 (C-9), 60.12 (C-8), 34.01(C-11), 32.50, 31.80, 29.56, 29.45, 29.37, 29.21, 29.08, 28.83 (C-12 to C-24), 24.76 (C-25), 22.58 (C-26), 18.00 (C-7), 13.98 (C-27). EI-MS: 433 [M^+](1), 151 (52), 123 (33), 83 (19), 69 (29), 55 (62), 43 (63), 41 (100). HR-MS: 433.3197 ($C_{26}H_{43}NO_4^+$; calc. 433.3192).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Cis,cis*-9,12-octadecadienoate (2e)*

As described for **2a**, but using *cis*,*cis*-9,12-octadecadienoic acid (1.1 ml, 3 mmol) as acylating agent: 311 mg (61%), viscous oil. IR (thin film, $CHCl_3$): 3448, 3024, 2938, 2857, 2704, 1741, 1394, 1226, 1160, 1039 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.86 (s, 1H, H-6), 5.35 (m, 4H, H-18, H-19, H-21 and H-22), 5.02 (s, 2H, H-9), 4.98 (s, 2H, H-8), 2.76 (m, 2H, H-20), 2.43 (s, 3H, H-7), 2.28 (t, 2H, J = 7.8 Hz, H-11), 2.04 (m, 4H, H-17 and H-23), 1.60 (m, 2H, H-12), 1.29 (m, 14H, H-13 to H-16 and H-24 to H-26), 0.89 (t, 3H, J = 6.9 Hz, H-27); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.22 (C-10), 152.14 (C-3), 148.08 (C-2), 138.90 (C-6), 131.03 (C-4), 130.12, 129.86, 128.02, 127.81 (C-18, C-19, C-21 and C-22), 126.35 (C-5), 61.11 (C-9), 60.08 (C-8), 34.01 (C-11), 31.42 (C-12), 29.48, 29.23, 28.99, 27.09, 25.55 (C-13 to C-17, C-20, C-23 and C-24), 24.75 (C-25), 22.46 (C-26), 18.00 (C-7), 13.95 (C-27). EI-MS: 431 [M^+](1), 151 (19), 123 (19), 106 (10), 81 (42), 67 (66), 55 (49), 43 (32), 41 (100). HR-MS: 431.3025 ($C_{26}H_{41}NO_4^+$; calc. 431.3036).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Cis,cis,cis*-9,12,15-octadecatrienoate (2f)*

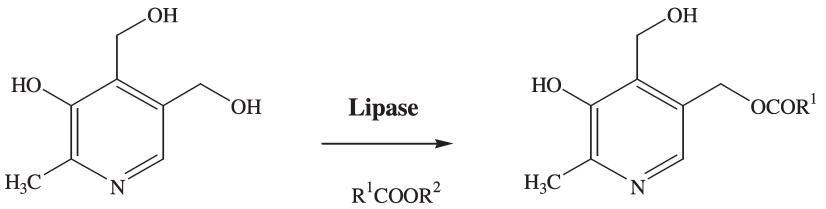
As described for **2a**, but using *cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid (1.1 ml, 3 mmol) as acylating agent: 310 mg (61%), viscous oil. IR (thin film, $CHCl_3$): 3447, 3009, 2938, 2852, 2695, 1747, 1469, 1227, 1163, 1042 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.84 (s, 1H, H-6), 5.32 (m, 6H, H-18, H-19, H-21, H-22, H-24 and H-25), 5.01 (s, 2H, H-9), 4.98 (s, 2H, H-8), 2.80 (m, 4H, H-20 and H-23), 2.43 (s, 3H, H-7), 2.28 (t, 2H, J = 7.8 Hz, H-11), 2.07 (m, 4H, H-17 and H-26), 1.59 (m, 2H, H-12), 1.28 (m, 8H, H-13 to H-16), 0.97 (t, 3H, J = 6.9 Hz, H-27); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.13 (C-10), 152.22 (C-3), 148.07 (C-2), 138.85 (C-6), 131.04 (C-4), 126.37 (C-5), 131.84, 130.09, 128.21, 128.13, 127.69, 127.05 (C-18, C-19, C-21, C-22, C-24 and C-25), 61.10 (C-9), 60.16 (C-8), 34.01 (C-11), 29.46, 29.00, 27.11, 25.47, 25.55, 24.77, 20.46 (C-12 to C-17, C-20, C-23 and C-26), 18.01 (C-7), 14.18 (C-27). EI-MS: 429 [M^+](3), 123 (20), 93 (21), 79 (56), 67 (60), 55 (61), 43 (38), 41 (100). HR-MS: 429.2882 ($C_{26}H_{39}NO_4^+$; calc. 429.2879).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Cis,cis,cis,cis*-5, 8, 11, 14-eicosatetraenoate (2g)*

As described for **2b**, but using *cis*,*cis*,*cis*,*cis*-5,8,11,14-eicosatetraenoic acid (1.2 ml, 3 mmol) as acylating agent: 318 mg (59%), viscous oil. IR (thin film, $CHCl_3$): 3445, 3010, 2930, 2855, 2687, 1751, 1415, 1226, 1152, 1024 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.95 (s, 1H, H-6), 5.37 (m, 6H, H-14, H-15, H-17, H-18, H-20, H-21, H-23 and H-24), 5.02 (s, 4H, H-8 and H-9), 2.80 (m, 6H, H-16, H-19 and H-22), 2.47 (s, 3H, H-7), 2.31 (t, 2H, J = 7.8 Hz, H-11), 2.07 (m, 4H, H-21 and H-30), 1.72 (m, 2H, H-12), 1.30 (m, 8H, H-17 to H-20), 0.88 (t, 3H, J = 6.9 Hz, H-31); ^{13}C-NMR ($CDCl_3$:CD_3OD) δ (ppm): 173.00 (C-10), 152.03 (C-3), 148.20 (C-2), 139.12 (C-6), 131.44 (C-4), 126.29 (C-5), 154.62, 130.93, 129.04, 128.58, 128.26, 127.96, 127.75, 127.45 (C-14 to C-16, C-18, C-20 to C-24), 61.21 (C-9), 60.18 (C-8), 33.45 (C-11), 31.45, 29.24, 27.17, 26.44, 25.55, 22.51 (C-12, C-13, C-16, C-19, C-22, C-25 to C-28), 18.09 (C-7), 13.99 (C-29). EI-MS: 455 [M^+](1), 123 (22), 91 (22), 81 (23), 79 (46), 67 (43), 55 (65), 43 (80), 41 (100). HR-MS: 455.3028 ($C_{28}H_{41}NO_4^+$; calc. 455.3036).

[5-Hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl] Methyl Cis*-13-docosenoate (2h)*

As described for **2b**, but using *cis*-9-octadecyl *cis*-13-docosenoate (2.1 ml, 3 mmol) as acylating agent: 323 mg (56%). M.p.: 60–61°C; IR (thin film, $CHCl_3$): 3437, 2924, 2859, 1726, 1469, 1248, 1162, 1035 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.90 (s, 1H, H-6), 5.34 (m, 2H, H-23 and H-24), 5.03 (s, 2H, H-9), 4.99 (s, 2H, H-8), 2.45 (s, 3H, H-7), 2.28 (t, 2H, J = 7.8 Hz, H-11), 2.01 (m, 4H, H-22 and H-25), 1.58 (m, 2H, H-12), 1.25 (m, 20H, H-13 to H-21 and H-26 to H-30), 0.88 (t, 3H, J = 6.9 Hz, H-31); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.30 (C-10), 151.73 (C-3), 148.45 (C-2), 139.67 (C-6), 130.42 (C-4), 129.88, 129.83 (C-23 and C-24), 126.19 (C-5), 61.21 (C-9), 60.29 (C-8), 34.12 (C-11), 31.88 (C-12), 29.75, 29.56, 29.48, 29.43, 29.29, 29.21, 29.11, 27.19, 27.17 (C-13 to C-22 and C-25 to C-28), 24.88 (C-29), 22.64 (C-30), 18.32 (C-7), 14.04 (C-31). EI-MS: 489 [M^+](2), 151 (100), 123 (53), 106 (16), 83 (21), 69 (33),



1 **2**

2a	n = 16	$R^1 = CH_3(CH_2)_nCO$-	$R^2 = CH_3CH_2$- or H
2b	n = 18	$R^1 = CH_3(CH_2)_nCO$-	R^2 = H
2c	--	$R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$- *cis*	R^2 = H
2d	--	$R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$- *trans*	R^2 = H
2e	--	$R^1 = CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7CO$- *cis, cis*	R^2 = H
2f	--	$R^1 = CH_3(CH_2CH{=}CH)_3(CH_2)_7CO$- *cis, cis, cis*	R^2 = H
2g	--	$R^1 = CH_3(CH_2)_3(CH_2CH{=}CH)_4\ (CH_2)_3CO$-	R^2 = H
2h	--	$R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_{11}CO$- *cis*	$R^2 = CH_3(CH_2)_7CH{=}CH(CH_2)_8$

SCHEME 1

55 (65), 43 (43), 41 (47). HR-MS: 489.3816 ($C_{26}H_{43}NO_4^+$; calc. 489.3818).

RESULTS AND DISCUSSION

We have prepared under mild reaction conditions monoacylated fatty acid derivatives of pyridoxine in a regioselective way and have obtained moderate to high yields. Various compounds have been obtained through acylation of the substrate using lipases from several sources as catalysts: porcine pancreas lipase (PPL), Lipozyme (LIP), *Candida rugosa* lipase (CRL) and *Candida antarctica* lipase (CAL). (Scheme 1).

The enzyme-catalyzed acylation produced, in a regioselective way, monoacylated derivatives with the acyl group exclusively suited in position 5 of the pyridine ring. See Table I.

We have tested the acylation reaction by using ethyl carboxylates as acylating agents as we did in our previous work on enzymatic acylation (Baldessari *et al.*, 1998). Carboxylic acids also gave good results, being more economical than ethyl esters. The erucic acid derivative **2h**, was prepared with oleyl erucate as an acylating agent. The oleyl alkyl chain did not change the regioselectivity, reaction conditions or yield of the enzymatic acylation, showing the capability of the lipase to perform its catalytic activity even with a long unsaturated alkyl chain in the acylating agent.

TABLE I Lipase-catalyzed acylation of pyridoxine **1**

Product	Enzyme	Time (h)	Yield (%)
2a	CAL	72	69
2a	LIP	120	59
2a	PPL	120	56
2a	CRL	120	42
2b	CAL	96	64
2c	CAL	72	60
2d	CAL	72	51
2e	CAL	72	61
2f	CAL	72	61
2g	CAL	96	59
2h	CAL	96	56

Reactions were performed under standard conditions.

Although the four enzymes were all regioselective CAL gave a higher yield and lower reaction time than the other three (Table I). Longer chain fatty acids required longer periods of reaction.

In order to optimize the reaction conditions, we have performed several experiments changing reaction parameters such as temperature, enzyme-substrate ratio (E/S) and acylating agent-substrate ratio (A/S). Under standard conditions, reactions were conducted at 30°C with an E/S and A/S ratio of 2.5/1. By using stearic acid as the acylating agent and acetone or acetonitrile as the solvent, at a higher temperature than standard conditions, such as 55°C, the reaction showed low regioselectivity. On the other hand, a twofold increase in acylating agent-substrate ratio did not show a higher yield in the reaction product.

The efficiency of the enzymes in acylation reactions was variable and decreased slightly as the chain length of the acylating agent increased. Increasing unsaturation had no significant effect on yield, but a remarkable yield dependence existed with *cis* and *trans* isomers of C_{18} carboxylic acids, with *cis* (oleic derivative) being higher than *trans*

TABLE II HLB values of fatty acid derivatives of pyridoxine

Compound	HLB*
Pyridoxine myristate	9.49
Pyridoxine stearate **2a**	8.27
Pyridoxine arachidate **2b**	7.77
Pyridoxine oleate **2c**	8.31
Pyridoxine elaydate **2d**	8.31
Pyridoxine linoleate **2e**	8.34
Pyridoxine linolenate **2f**	8.39
Pyridoxine arachidonate **2g**	8.43
Pyridoxine erucate **2h**	7.35

*Data calculated with physical properties! Pro™ revision 2.4

(elaidic derivative). The regioselectivity was retained in every case.

The solvent effect was also studied. Attempts to use nonpolar solvents such as hexane, toluene and methylene chloride and solvent free systems at 30 and 50°C were not effective. Due to their highly hydroxylated nature, pyridoxine is only soluble in polar solvents such as acetonitrile and acetone. The lipase-catalyzed reaction in acetone afforded the best results. Without enzymes pyridoxine did not react at all.

Novel products obtained were completely identified by spectroscopic methods: FTIR, ^{1}H and ^{13}C NMR and HR-MS.

Compounds **2a**–**h** have not been previously reported. Their structure with a hydrophilic moiety from pyridoxine and a long nonpolar one from the fatty acid, led us to consider that these products should exhibit surfactant activity. Surfactants are characterized according to the balance between the hydrophilic (polar) and lipophilic (non polar) portions of their molecules. The hydrophilic–lipophilic balance (HLB) number indicates the polarity of the molecules in an arbitraty range of 1–40, with the most commonly used emulsifiers having a value between 1 and 20 (Griffin, 1949). Surfactants with a strong lipophilic character have a lower HLB, while the ones with a stronger hydrophilic value have a higher HLB. The HLB value is obtained by dividing the molecular weight of the water-soluble portion of the molecule by the total molecular weight and multiplying the result by 20. The HLB value is a useful guide to select an emulsifier for a given application. Emulsifiers having a low HLB, typically around 4, are suitable for water-in-oil (W/O) emulsions and the ones having intermediate to high HLB are suitable for oil-in-water (O/W) emulsions. Calculation of the hydrophilic–lipophilic balance HLB (Chem SW™, Inc., 1997) of **2a**–**h** and pyridoxine myristate (Baldessari and Mangone, 2000) gave values corresponding to O/W emulsifiers (Becher, 1966). Table II shows the results.

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