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Lipase-Catalyzed Preparation of Biologically Active Esters of Dehydroepiandrosterone

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A series of acyl esters, derivatives of dehydroepiandrosterone have been prepared by an enzymatic methodology. The acyl chain had a length that varied from two to eighteen carbon atoms. The C_{18} derivative could be saturated or unsaturated. Following this biocatalytic approach we have also obtained a chloropropionyl derivative. We have observed that several lipases catalyzed esterification and transesterification reactions of dehydroepiandrosterone with carboxylic acids or alkyl carboxylates. The advantages presented by this methodology such as mild reaction conditions, economy and low environmental impact, make biocatalysis a convenient way to prepare acyl derivatives of DHEA with biological activity.

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Keywords: lipase-catalyzed; Enzymatic acylation; Dehydroepiandrosterone

INTRODUCTION

Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one; hereafter DHEA) is one of the most abundant steroids in human blood. It is a secretory product of the human adrenal cortex. Serum DHEA concentrations decrease with age (Orentreich *et al.*, 1992) which has led to speculation regarding the possible role of the DHEA hormone in aging (Allolio and Arlt, 2002).

In humans, DHEA is an intermediate product in the biosynthesis of sex steroid hormones such as androgens and estrogens (Arlt *et al.*, 1999) and it acts as a neurosteroid, affecting neurotransmitter receptors in the brain. DHEA has a considerable effect in mood disorders, well-being and sexuality of patients with adrenal insufficiency. (Wolkowitz *et al.*, 1993). Despite studies demonstrating a neuromodulatory action of DHEA in the brain, the precise physiological and pathophysiological roles and the corresponding mechanisms of these neurosteroids are still largely unknown. It has been reported that DHEA and their fatty acid ester derivatives are more abundant in the brain than in other tissues (Jo *et al.*, 1989). As a consequence of their high lipophilicity these compounds could modify membrane function and structure or interact with specific cell-surface proteins (Di Paolo, 1994).

Many therapeutically useful steroids are administered as esters with the widespread distribution of the esterase activity in mammalian tissue ensuring the cellular availability of the free steroid (Lund-Pero *et al.*, 1994). Since the fatty acid esters of DHEA are very lipophilic and have long half-lives, they would be expected to concentrate in fat, where release of free DHEA by action of an esterase could enhance the availability of DHEA in target tissues (Xu *et al.*, 2002). Therefore, DHEA aliphatic esters could be useful to ascertain the possible utility of DHEA in therapy and to provide standards for metabolic studies.

Following our work on lipase-catalyzed acylation and deacylation of steroids and vitamin B_6 (Baldessari *et al*., 1995, 1996, 1998; Baldessari and Mangone, 2002), the present paper reports results obtained in the preparation of esters of DHEA by enzymatic methodology.

The synthesis of some aliphatic esters of DHEA containing saturated chains of 2, 4, 12, 14 and 16 carbons by a conventional chemical procedure has been reported (Lardy *et al.*, 1998). The reagents used such as acid anhydrides or chlorides and pyridine are not friendly to the environment (http://msdssearch.com). The enzymatic methodology, involving environmentally acceptable conditions of Green Chemistry, has been applied in this work and resulted in the production of eleven esters of DHEA with alkyl chains containing from two to eighteen carbon atoms. An unsaturated fatty acid

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and chloroacid derivative were also prepared using this enzymatic approach.

MATERIALS AND METHODS

Materials

All solvents and reagents were of analytical grade. Dehydroepiandrosterone (DHEA), ethyl propionate, ethyl butyrate, carboxylic acids, and lipase from *Candida rugosa* (905 U mg^{-1} solid), were purchased from Sigma Chemical Co.; *Candida antarctica* lipase B: Chirazyme L-2, c.-f., C3, lyo (400 U g^{-1}) was purchased from Roche Diagnostics GmbH; *Pseudomonas* lipase: Lipase PS-C Amano II (804 U g^{-1}) and Lipase PS Amano (33,200 Ug^{-1}) were purchased from Amano Pharmaceutical Co. All enzymes were used as supplied.

Analytical

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. Analytical GC was performed using a Hewlett Packard-5890 gas chromatograph with a HP-5 capillary column (50 m × 0.32 mm) and HP-17 (10 m × 0.53 mm). ^{1}H-NMR and ^{13}C-NMR spectra were measured on Bruker AC-200 and Bruker AM- 500 spectrometers. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) as internal standard, using $CDCl_3$ as solvent. Assignments were based on DEPT spectra and by comparison with reported data. Carbon atoms are arbitrarily numbered from 1 to 19 in the steroid skeleton and from 1′ to 18′ in the acyl chain for NMR assignments. Mass spectra were determined on a HP-MS 1100, using electrospray ionization.

Enzymatic Preparation

3β-Acetoxy-5-androsten-17-one (2a)

One g of CRL was added to a solution of **1** (172 mg, 0.6 mmol) in ethyl acetate (25 ml). The suspension was stirred (200 rpm) at 30°C and the progress of reaction monitored by TLC (CH_2Cl_2:CH_3OH (95:5)) and GC. After the indicated time, the enzyme was filtered off, the solvent evaporated, and the crude residue purified by silica gel chromatography (dichloromethane): 194 mg (98%) of **2a**. ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.2Hz); 4.61 (m, 1H, H-3); 2.03 (s, 3H, H-2′); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.95 (C-1); 27.70 (C-2); 73.72 (C-3); 38.09 (C-4); 139.90 (C-5); 121.86 (C-6); 31.43 (C-7); 31.50 (C-8); 50.17 (C-9); 36.70 (C-10); 20.33 (C-11); 30.77 (C-12); 47.40 (C-13); 51.72 (C-14); 21.88 (C-15); 35.83 (C-16); 220.40 (C-17); 13.55 (C-18); 19.33 (C-19); 21.40 (C-2′); 170.40 (C-1′). ESI-MS *m/z* (relative intensity): 353 [$(M+Na)^+$, 70], 271 [$(DHEA+H)^+$, 100].

3β-Propoxy-5-androsten-17-one (2b)

One g of CRL was added to a solution of **1** (172 mg, 0.6 mmol) in toluene (25 ml) containing ethyl propionate (100 μl, 1.5 mmol), or propionic acid (70 μl, 1.5 mmol). The suspension was stirred (200 rpm) at 30°C and the progress of reaction was monitored by TLC (CH_2Cl_2:CH_3OH (95:5)) and GC. After the indicated time, the enzyme was filtered off, the solvent evaporated, and the crude residue purified by silica gel chromatography (dichloromethane): 198 mg (99%) from ethyl propionate and 154 mg (77%) from propionic acid, of **2b**. ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.2Hz); 4.62 (m, 1H, H-3); 1.13 (t, 3H, H-3′, J = 7.7 Hz); 2.28 (c, 2H, H-2′, J = 7.7 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.93 (C-1); 27.72 (C-2); 73.46 (C-3); 38.10 (C-4); 139.93 (C-5); 121.77 (C-6); 31.39 (C-7); 31.46 (C-8); 50.12 (C-9); 36.73 (C-10); 20.31 (C-11); 30.77 (C-12); 47.51 (C-13); 51.68 (C-14); 21.88 (C-15); 35.84 (C-16); 221.04 (C-17); 13.54 (C-18); 19.35 (C-19); 27.91 (C-2′-); 9.19 (C-3′); 173.90 (C-1′). ESI-MS *m/z* [relative intensity]: 345 $[(M+H)^+$, 5], 367 $[(M+Na)^+$, 90], 383$[(M+K)^+$, 10], 288 $[(M\text{-}CH_3CO+H)^+]$, 271 $[(DHEA+H)^+$, 100].

3β-(3-Chloropropoxy)-5-androsten-17-one (2c)

As described for **2b**, but using ethyl 3-chloropropionate (120 μl, 1.5 mmol) as acylating agent: 225 mg (99%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.67 (m, 1H, H-3); 3.76 (t, 2H, H-3′, J = 6.7 Hz); 2.77 (t, 2H, H-2′, J = 6.7 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.87 (C-1); 27.65 (C-2); 74.44 (C-3); 37.99 (C-4); 139.71 (C-5); 122.05 (C-6); 31.40 (C-7); 31.45 (C-8); 50.11 (C-9); 36.72 (C-10); 20.33 (C-11); 30.77 (C-12); 47.52 (C-13); 51.69 (C-14); 21.87 (C-15); 35.84 (C-16); 221.00 (C-17); 13.55 (C-18); 19.34 (C-19); 39.18 (C-3′); 37.87 (C-2′); 170.40 (C-1′). ESI-MS *m/z* [relative intensity]: 379 $[(M+H)^+$, 5], 401 $[(M+Na)^+$, 90], 417$[(M+K)^+$, 10], 396 $[(M+H_2O)^+$, 45], 288 $[M\text{-}CH_3CO+H)^+$, 5], 271 $[(DHEA+H)^+$, 100].

3β-Butyroxy-5-androsten-17-one (2d)

As described for **2b**, but using ethyl butyrate (120 μl, 1.5 mmol) as acylating agent: 182 mg (85%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.2Hz); 4.62 (m, 1H, H-3); 0.95 (t, 3H, H-4′, J = 7.4 Hz); 1.65 (m, 2H, H-3′); 2.26 (t, 2H, H-2′, J = 7.4 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.95 (C-1); 27.75 (C-2); 73.41 (C-3); 38.13 (C-4); 139.95 (C-5); 121.78 (C-6); 31.42 (C-7); 31.47

(C-8); 50.13 (C-9); 36.74 (C-10); 20.34 (C-11); 30.79 (C-12); 47.53 (C-13); 51.71 (C-14); 21.90 (C-15); 35.86 (C-16); 221.01 (C-17); 13.56 (C-18); 19.37 (C-19); 13.66 (C-4′); 18.55 (C-3′); 26.85 (C-2′); 173.09(C-1′). ESI-MS *m/z* [relative intensity]: 381 $[(M+Na)^+$, 45], 376 $[(M+H_2O)^+$, 35], 288 $[M\text{-}CH_3CO+H)^+$, 5], 271 $[(DHEA+H)^+$, 100].

3β-Hexanoyloxy-5-androsten-17-one (2e)

As described for **2b**, but using hexanoic acid (130 μl, 1.5 mmol) or ethyl hexanoate (140 μl, 1.5 mmol) as acylating agent: 213 mg (95%) from hexanoic acid and 215 mg (96%) from ethyl hexanoate. ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.61 (m, 1H, H-3); 0.90 (t, 3H, H-6′, J = 7.0 Hz); 2.27 (t, 2H, H-2′, J = 7.5 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.90 (C-1); 27.69 (C-2); 73.37 (C-3); 38.07 (C-4); 139.92 (C-5); 121.79 (C-6); 31.35 (C-7); 31.40 (C-8); 50.06 (C-9); 36.70 (C-10); 20.27 (C-11); 30.73 (C-12); 47.50 (C-13); 51.63 (C-14); 21.85 (C-15); 35.83 (C-16); 221.04 (C-17); 13.52 (C-18); 19.34 (C-19); 13.94 (C-6′); 22.3, 31.27, 24.72, 34.63 (C-2′ to C-5′)173.33 (C-1′). ESI-MS *m/z* [relative intensity]: 409 $[(M+Na)^+$, 90], 425 $[(M+K)^+$, 10], 271 $[(DHEA+H)^+$, 100].

3β-Dodecanoyloxy-5-androsten-17-one (2f)

As described for **2b**, but using dodecanoic acid (300 mg, 1.5 mmol) as acylating agent: 254 mg (90%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.62 (m, 1H, H-3); 0.88 (t, 3H, H-12′, J = 7.0 Hz); 2.27 (t, 2H, H-2′, J = 7.5 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.92 (C-1); 27.72 (C-2); 73.41 (C-3); 38.10 (C-4); 139.96 (C-5); 121.79 (C-6); 31.37 (C-7); 31.44 (C-8); 50.10 (C-9); 36.71 (C-10); 20.30 (C-11); 30.76 (C-12); 47.52 (C-13); 51.67 (C-14); 21.86 (C-15); 35.84 (C-16); 221.04 (C-17); 13.52 (C-18); 19.34 (C-19); 14.11 (C-12); 22.67, 33.93, 29.58, 29.41, 29.22, 29.05, 29.09, 29.32, 25.05, 34.68 (C-2′ to C-11′); 173.35 (C-1′). ESI-MS *m/z* [relative intensity]: 493 $[(M+Na)^+$, 20], 509$[(M+K)^+$, 10], 483 $[(M+H_2O)^+$, 50], 271 $[(DHEA+H)^+$, 100].

3β-Hexadecanoyloxy-5-androsten-17-one (2g)

As described for **2b**, but using hexadecanoic acid (384 mg, 1.5 mmol) as acylating agent: 274 mg (87%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.40 (d, 1H, H-6, J = 5.2Hz); 4.62 (m, 1H, H-3); 0.88 (t, 3H, H-16′, J = 6.9 Hz); 2.27 (t, 2H, H-2′, J = 7.5 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.93 (C-1); 27.73 (C-2); 73.39 (C-3); 38.11 (C-4); 139.93 (C-5); 121.77 (C-6); 31.39 (C-7); 31.46 (C-8); 50.11 (C-9); 36.73 (C-10); 20.31 (C-11); 30.78 (C-12); 47.50 (C-13); 51.68 (C-14); 21.83 (C-15); 35.84 (C-16); 221.10 (C-17); 13.54 (C-18); 19.35 (C-19); 14.14 (C-16′); 22.70, 31.92, 29.70, 29.66, 29.60, 29.45, 29.36, 29.24, 29.10, 25.06, 34.69 (C-2′ to C-15′); 173.28 (C-1′). ESI-MS *m/z* [relative intensity]: 549 $[(M+Na)^+$, 10], 565$[(M+K)^+$, 10], 544 $[(M+H_2O)^+$, 100], 288 $[M\text{-}CH_3CO+H)^+$, 5], 271 $[(DHEA+H)^+$, 35].

3β-Octadecanoyloxy-5-androsten-17-one (2h)

As described for **2b**, but using octadecanoic acid (426 mg, 1.5 mmol) as acylating agent: 329 mg (99%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.62 (m, 1H, H-3); 0.88 (t, 3H, H-18′, J = 7.0 Hz); 2.27 (t, 2H, H-2′, J = 7.5 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.95 (C-1); 27.75 (C-2); 73.41 (C-3); 38.11 (C-4); 139.95 (C-5); 121.78 (C-6); 31.40 (C-7); 31.47 (C-8); 51.69 (C-9); 36.74 (C-10); 20.33 (C-11); 30.79 (C-12); 47.52 (C-13); 51.69 (C-14); 21.90 (C-15); 35.86 (C-16); 221.12 (C-17); 13.56 (C-18); 19.37 (C-19); 14.15 (C-18′); 22.71, 31.94, 29.71, 29.67, 29.61, 29.48, 29.38, 29.26, 29.11, 29.07, 25.07, 34.71 (C-2′ to C-17′); 173.32 (C-1′). ESI-MS *m/z* [relative intensity]: 577 $[(M+Na)^+$, 18], 593$[(M+K)^+$, 5], 572 $[(M+H_2O)^+$, 25], 288 $[M\text{-}CH_3CO+H)^+$, 10], 271 $[(DHEA+H)^+$, 100].

3β-cis-9-Octadecenoyloxy5-androsten-17-one (2j)

As described for **2b**, but using *cis*-9-octadecenoic acid (0.6 ml, 1.5 mmol) as acylating agent: 265 mg (80%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.62 (m, 1H, H-3); 0.88 (t, 3H, H-18′, J = 7.0 Hz); 5.33 (m, 2H, H-9′ and H-10′); 2.00 (m, 4H, H-8′ and H-11′); 2.28 (t, 2H, H-2′, J = 7.8 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.91 (C-1); 27.70 (C-2); 73.38 (C-3); 38.08 (C-4); 139.93 (C-5); 121.80 (C-6); 31.36 (C-7); 31.43 (C-8); 50.08 (C-9); 36.70 (C-10); 20.29 (C-11); 30.75 (C-12); 47.52 (C-13); 51.65 (C-14); 21.86 (C-15); 35.84 (C-16); 221.10 (C-17); 13.52 (C-18); 19.34 (C-19); 14.14 (C-18′); 22.67 (C-17′), 31.89 (C-16′), 29.76, 29.68, 29.58, 29.45, 29.36, 29.24, 29.14, 29.08, (C-4′ to C-6′ and C-12′ to C-15′); 27.20, 27.14 (C-8′ and C-11′); 129.74, 129.93 (C-9′ and C-10′); 25.05 (C-3′), 34.68 (C-2′); 173.30 (C-1′). ESI-MS *m/z* [relative intensity]: 575 $[(M+Na)^+$, 15], 591$[(M+K)^+$, 15], 570 $[(M+H_2O)^+$, 100], 288 $[M\text{-}CH_3CO+H)^+$, 20], 271 $[(DHEA+H)^+$, 38].

3β-cis,cis-9,12-Octadecadienoyloxy-5-androsten-17-one (2k).

As described for **2b**, but using *cis,cis*-9,12-octadecadienoic acid (0.6 ml, 3 mmol) as acylating agent: 259 mg (78%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.62 (m, 1H, H-3); 0.89 (t, 3H, H-18′, J = 7.0 Hz); 5.35 (m, 4H, H-9′ and H-10′); 2.77 (t, 2H, H-11′, J = 6.5 Hz); 2.05 (m, 4H, H-8′ and H-14′); 2.27 (t, 2H, H-2′, J = 7.5 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.92 (C-1);

27.72 (C-2); 73.41 (C-3); 38.10 (C-4); 139.95 (C-5); 121.80 (C-6); 31.39 (C-7); 31.48 (C-8); 50.10 (C-9); 36.72 (C-10); 20.31 (C-11); 30.76 (C-12); 47.52 (C-13); 51.68 (C-14); 21.87 (C-15); 35.84 (C-16); 221.10 (C-17); 13.54 (C-18); 19.34 (C-19); 14.08 (C-18′); 22.57 (C-17′), 130.19, 130.03, 128.00, 127.87 (C-9′, C-10′, C-12′ and C-13′); 25.61 (C-11′); 25.02 (C-3′)), 34.67 (C-2′); 27.18 (C-8′); 31.5, 31.45, 31.41, 29.58, 29.33, 29.16, 29.09 (C-4′ to C-7′); 173.30 (C-1′). ESI-MS *m/z* [relative intensity]: 573 [$(M+Na)^+$, 38], 589[$(M+K)^+$, 5], 568 [$(M+H_2O)^+$, 100], 288 [M-$CH_3CO+H)^+$, 58], 271 [$(DHEA+H)^+$, 90].

3β-cis,cis,cis-9,12,15-Octadecatrienoyloxy-5-androsten-17-one (2m).

As described for **2b**, but using *cis,cis,cis*-9,12,15-octadecatrienoic acid (0.6 ml, 1.5 mmol) as acylating agent: 315 mg (96%). ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.41 (d, 1H, H-6, J = 5.1Hz); 4.62 (m, 1H, H-3); 0.98 (t, 3H, H-18′, J = 7.6 Hz); 5.36 (m, 6H, H-9′, H-10′, H-12′, H-13′, H-15′ and H-16′); 2.82 (t, 4H, H-11′ and H-16′, J = 6.1Hz); 2.07 (m, 4H, H-17′); 2.27 (t, 2H, H-2′, J = 7.6 Hz); 1.05 (s, 3H, H-19); 0.89 (s, 3H, H-18). ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.90 (C-1); 27.71 (C-2); 73.39 (C-3); 38.08 (C-4); 139.89 (C-5); 121.80 (C-6); 31.36 (C-7); 31.43 (C-8); 50.08 (C-9); 36.70 (C-10); 20.30 (C-11); 30.75 (C-12); 47.51 (C-13); 51.65 (C-14); 21.87 (C-15); 35.84 (C-16); 221.13 (C-17); 13.53 (C-18); 19.35 (C-19); 14.30 (C-18′); 20.54 (C-17′), 130.26, 131.91, 128.22, 128.19, 127.65, 127.04 (C-9′, C-10′, C-12′, C-13′, C-15′ and C-16′); 25.59, 25.51 (C-11′ and C-14′); 25.08 (C-3′), 34.66 (C-2′); 27.18 (C-7′); 29.55, 29.06, 29.16, 29.09 (C-4′ to C-8′).; 173.30 (C-1′). ESI-MS *m/z* [relative intensity]: 571 [$(M+Na)^+$, 100], 587 [$(M+K)^+$, 5], 566 [$(M+H_2O)^+$, 5], 288 [M-$CH_3CO+H)^+$, 60], 271 [$(DHEA+H)^+$, 85].

RESULTS AND DISCUSSION

We have prepared acyl derivatives of DHEA in high to quantitative yield under mild conditions using an enzymatic methodology. Compounds **2a–m** were obtained by acylation of the substrate using lipases from several sources such as *Candida rugosa* (CRL), *Candida antarctica* (CAL), lipase PS (PSL) and PS-C (PSL-C) from *Pseudomonas* sp. (Scheme 1).

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The four commercial lipases previously mentioned were tested in the acylation of DHEA **1** with ethyl acetate to obtain **2a,** and with hexadecanoic acid to obtain **2g**.

As can be seen from Table I, the lipase from *Candida rugosa* (CRL) gave the most satisfactory results in both cases. PSL and PSL-C were also active but showed a lower performance. CAL was effectively inert in the reaction with ethyl acetate and ethyl hexadecanoate at 30°C or 55°C. These results are in accordance with the regioselective behavior of lipases in the acylation and deacylation of steroids (Baldessari *et al.*, 1995, 1996; Bruttomesso and Baldessari, 2003). Without enzymes DHEA did not react at all.

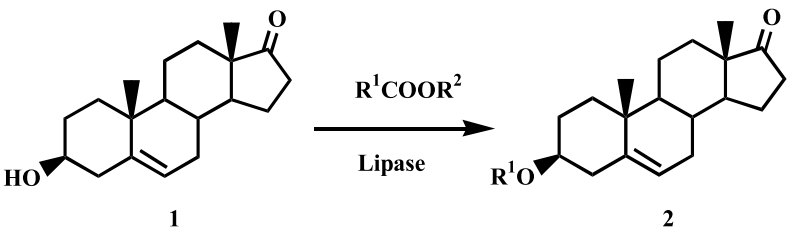
As in our previous studies on enzymatic acylation (Baldessari *et al.*, 1998), in the present work we tested the acylation reaction by using ethyl carboxylates as acylating agents in the case of short chain length derivatives (**2a–e**).

Table II shows that the products were obtained in high yield and it was not necessary to use activated esters to perform the enzymatic transesterification reaction (Bertinotti *et al.*, 1994). Carboxylic acids also gave good results, in particular in the preparation of long chain derivatives, which would be even more economic than ethyl esters. The efficiency of acylation reactions was variable showing no correlation between the product yield and the increasing chain length of the acylating agent.

Some of these compounds have been previously prepared according to the known chemical procedures by using acid anhydrides or chlorides (Lardy *et al.*, 1998). These reagents are moisture sensitive, corrosive and lachrymators (http://msdssearch.com) and the acylation reaction must be performed in pyridine and sometimes under an inert gas atmosphere. The enzymatic approach is simple and only uses ethyl carboxylates or carboxylic acids as acylating agents, which are readily available and less expensive.

To optimize the conditions of the reaction we have performed several experiments changing reaction parameters such as temperature, enzyme/substrate ratio (E/S) and acylating agent/substrate ratio (A/S). Table II shows the results obtained for a CRL-catalyzed reaction at 30°C and 55°C respectively. It can be concluded that where the yield was not already high at 30°C an increase in the temperature of the reaction did not improve the results and in the case of longer unsaturated chains, such as oleic (**2j**), linoleic (**2k**) and linolenic (**2m**) derivatives, an increase in temperature decreased the yield of the reaction. The influence of the E/S ratio is showed in Table III. It can be observed that for a 72 h reaction period an E/S ratio or 5:1 gave the best results in the CRL-catalyzed reaction of DHEA and ethyl hexanoate.

Finally we have investigated the effect of the ratio of acylating agent and DHEA with ethyl acetate and ethyl hexanoate as acylating agents and CRL as biocatalyst and noticed that the proportion of acylating agent had a slight effect on the product yield. Table IV shows the degree of conversion obtained with CRL at different ratios. An acylating A/S ratio of 2.5/1 was the best for a reaction period of 72 h.



1 2

2a $R^1 = CH_3CO-$ (CH_3: 2′; CO: 1′) $R^2 = CH_3CH_2-$

2b $R^1 = CH_3CH_2CO-$ (CH_3: 3′; CO: 1′) $R^2 = CH_3CH_2-$ or H

2c $R^1 = ClCH_2CH_2CO-$ $R^2 = CH_3CH_2-$

2d $R^1 = CH_3(CH_2)_2CO-$ $R^2 = CH_3CH_2-$

2e $R^1 = CH_3(CH_2)_4CO-$ $R^2 = CH_3CH_2-$ or H

2f $R^1 = CH_3(CH_2)_{10}CO-$ $R^2 = H$

2g $R^1 = CH_3(CH_2)_{14}CO-$ $R^2 = H$

2h $R^1 = CH_3(CH_2)_{16}CO-$ (18′; 1′) $R^2 = H$

2j $R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CO-$ *cis* (18′; 10′; 9′; 1′) $R^2 = H$

2k $R^1 = CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7CO-$ *cis, cis* (18′; 14′ 13′ 12′; 11′ 10′ 9′) $R^2 = H$

2m $R^1 = CH_3(CH_2CH{=}CH)_3(CH_2)_7CO-$ *cis, cis, cis* (18′; 17′ 16′ 15′; 14′ 13′ 12′; 11′ 10′ 9′) $R^2 = H$

SCHEME 1

Considering the studies previously mentioned we have chosen as standard conditions a temperature of 30°C, an E/S ratio of 5/1 and A/S ratio of 2.5/1. The effect of the solvent was also studied. Attempts to use polar solvents such as acetonitrile, dioxane and tetrahydrofurane were not effective and CRL showed a poor activity giving only 20%, 34% and 21% of acylation product of DHEA with ethyl hexanoate respectively. The highest activity of CRL was displayed in toluene, so this solvent was chosen to obtain the products **2b** to **2m**. In the case of **2a**, ethyl acetate acted both as solvent and acylating agent.

TABLE I Effect of lipases from several sources on the acylation of DHEA **1.**

Product	Enzyme	Acylating agent	Time (h)	Yield (%)[a]
2a	CRL	ethyl acetate	72	98
2a	CAL	ethyl acetate	120	8
2a	PSL	ethyl acetate	120	15
2a	PSL-C	ethyl acetate	120	30
2g	CRL	hexadecanoic acid	72	96
2g	CAL	hexadecanoic acid	120	4
2g	PSL	hexadecanoic acid	120	5
2g	PSL-C	hexadecanoic acid	120	27

[a] Reactions were performed under standard conditions.

TABLE II CRL-catalyzed acylation of DHEA **1**. Effect of temperature.

Product	Time (h)	Acylating agent	Yield (%)[a,b] 30°C	55°C
2a	72	ethyl acetate	98	97
2b	72	ethyl propionate	99	99
2b	72	propionic acid	77	78
2c	72	ethyl chloropropionate	99	98
2d	72	ethyl butyrate	85	85
2e	72	ethyl hexanoate	96	97
2e	72	hexanoic acid	95	98
2f	72	dodecanoic acid	90	89
2g	72	hexadecanoic acid	87	88
2h	72	octadecanoic acid	99	97
2j	96	*cis*-9-octadecenoic acid	80	73
2k	96	*cis,cis*-9,12-octadecadienoic acid	78	70
2m	96	*cis,cis,cis*-9,12,15-octadecatrienoic acid	96	85

[a] Isolated product, purified by column chromatography.
[b] Reactions were performed under standard conditions.

TABLE III Enzyme:substrate ratio on the CRL-catalyzed acylation of DHEA **1** with ethyl hexanoate.

E/S	Yield (%)[a]
1:1	57
2.5:1	69
5:1	97
10:1	98
20:1	95

[a] Determined by GLC. Reactions were performed under standard conditions for 72 h.

TABLE IV Acylating agent:substrate ratio on the CRL-catalyzed acylation of DHEA **1.**

	Yield (%)[a]	
A/S	Ethyl acetate	Ethyl hexanoate
1:1	80	77
2.5:1	99	96
5:1	97	95
10:1	98	98
20:1	96	97

[a] Determined by GLC. Reactions were performed under standard conditions for 72 h.

CONCLUDING REMARKS

This work describes the application of enzymes for the preparation of a series of acyl derivatives of dehydroepiandrosterone. Lipases from different sources exhibited different performance as catalysts in the acylation reaction. *Candida rugosa* lipase gave the best results. By enzymatic acylation we have obtained various derivatives **2a–m** of dehydroepiandrosterone, some of which have not previously been reported in the literature. These products can be useful as potential therapeutic agents to elucidate the properties of DHEA and provide standards for metabolic studies.

The advantages presented by this methodology are: mild reaction conditions, economy and low environmental impact. Moreover, ethyl carboxylates and carboxylic acid, which are considered inert under these conditions in traditional chemical methodology, gave excellent results as acylating agents.

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