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A facile synthesis of an indol-dihydrotestosterone succinate derivative

Lauro Figueroa-Valverde[1], Francisco Díaz-Cedillo[2], Elodia García-Cervera[1]

[1]*Lab. Farmacoquímica, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Campeche, Av. Agustín Melgar, Col Buenavista C.P.24039 Campeche Cam., México.*
[2]*Esc. Nal. de Ciencias Biológicas del Instituto Politécnico Nacional. Prol. Carpio y Plan de Ayala s/n Col. Santo Tomas, México, D.F. C.P. 11340.*

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In this study, an indol-dihydrotestosterone succinate derivative (**4**) was synthesized. The first stage, involved the preparation of an indol-dihydrotestosterone derivative (**3**) by the reaction of dihydrotestosterone (**1**) with phenylhydrazine using hydrochloric acid as catalyst. The second stage was achieved by reacting **3** with anhydride succinic or succinic acid to form an indol-dihydrotestosterone succinate derivative (**4**).

Keywords: dihydrotestosterone, phenylhydrazine, succinate.

INTRODUCTION

There are several methods for the synthesis de indoles; for example, the preparation of 2-Substituted Indoles by Photostimulated Reactions of *o*-Iodoanilines with Carbanions[1]. Other reports show the preparation of a series of benzonitrile derivatives on position 6 or 4 of indole ring via a Leimgruber-Batcho reaction[2]. In addition, other studies showed that ruthenium catalyzed synthesis of indoles from N-substituted anilines and alkanolamines[3]. Other data show the synthesis of 2-substituted indoles from 2-ethynylanilines with tetrabutylammonium fluoride[4]. It is important to mention, that has been development several indoles steroid derivatives, for example, the synthesis of 17-indazole androstene derivatives[5] using dehydroepiandrosterone acetate as substrate. Other data showed the procedure for synthesis of 1'-Methylindolo (3',2':2, 3)2(5a)-androsten-17-one which was prepared by the Fischer indole synthesis[6]. Additionally, other studies show the synthesis of 1′*H*-5α-Cholest-2-eno[3,2–*b*]indoles using the Fisher reaction[7]. All these works show several procedures are available for synthesis of several indol-compounds derivatives; nevertheless, expensive reagents and special conditions are required. In this study, an indol-dihydrotestosterone succinate derivative (**4**) was synthetized; the first stage was achieved by reacting dihydrotestosterone (**1**) with phenylhydrazine (**2**) in presence of hydrochloric acid to form 3-indole-dihydrotestosterone derivative (**3**); the second stage was achieved by reacting **3** with anhydride succinic or succinic acid to form **4**.

EXPERIMENTAL

General methods

Dihydrotestosterone and the other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting points for the different compounds were determined on an Electrothermal (900 model). Infrared spectra (IR) were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR–300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

10,13-Dimethyl-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-8-aza-inden [2,3,b]cyclo-penta[a]phenanthren-17-ol (3).

A solution of 100 mg dihydrotestosterone (0.34 mmol), 60 mg phenylhydrazine (0.55 mmol) in 10 mL of ethanol was stirred for 10 min at room temperature. Then 0.5 mL of hydrochloric acid was added and the mixture was stirred for 48 h at room temperature. The reaction mixture was evaporated to a smaller volume, diluted with water, and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure, the residue was purified by crystallization from methanol-water (3:1) yielding 75% of product;

* To whom all correspondence should be sent:
E-mail: lauro_1999@yahoo.com

m.p.: 192-194 ºC; IR: V_{max} = 3,330, 3119 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H; 0.76 (s, 3H), 0.78 (s, 3H), 0.98–1.13 (m, 4H), 1.30–1.46 (m, 6H), 1.55-1.93 (m, 7H), 2.23–2.61 (m, 4H), 4.01 (m, 1H), 7.09 (m, 1H), 7.18 (m, 1H), 7.36 (m, 1H), 7.42 (m, 2H), 7.49 (broad, 2H) ppm. ^{13} C NMR (75.4 Hz, $CDCl_3$) δ_C; 11.12 (**C**–26), 12.40 (**C**–25), 20.60 (**C**–16), 23.83 (**C**–20), 24.05 (**C**–24), 29.48 (**C**–12), 31.02 (**C**–21), 31.96 (**C**–13), 33.68 (**C**-9), 35.60 (**C**–14), 37.03 (**C**–17), 37.79 (**C**–10), 40.55 (**C**–11), 43.93 (**C**-18), 51.44 (**C**–19), 54.76 (**C**–15), 76.86 (**C**–22), 105.72 (**C**–3), 110.56 (**C**–8), 117.63 (**C**-6), 118.21 (**C**–5), 120.79 (**C**–4), 126.65 (**C**–7), 133.72 (**C**–23), 135.33 (**C**–2) ppm. EI-MS *m/z:* 363.16 (M^+ 13), 237.34, 129.16. Anal. Calcd for $C_{25}H_{33}NO$: C, 82.60; H, 9.15, N, 3.85; O, 4.44. Found: C, 82.60; H, 9.12

Succinic acid mono-(10, 13-Dimethyl-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradeca-hydro-1H-8-aza-inden[2,3,b]cyclopenta[a]phenanthren-17-yl)ester (4).

Method A

The compound **3** (100 mg, 0.27 mmol) was added to a solution of 54 mg anhydride succinic (0.54 mmol), 3 mL of pyridine in 10 mL of toluene was gently refluxed for 8 h, and then cooled to room temperature. The reaction mixture was evaporated to a smaller volume, diluted with water, and extracted with chloroform. The organic phase was evaporated to dryness under reduced pressure. The residue was purified by crystallization from hexane:methanol:water (1:2:1) yielding 78% of product **4**. m.p.: 158–160 ºC; IR: V_{max} = 3,326, 1,615, 1,712 cm-1; ^1H NMR (300 MHz, $CDCl_3$) δ_H; 0.78 (s, 3H), 0.80 (s, 3H), 1.01–1.14 (m, 4H), 1.30-1.46 (m, 3H), 1.51–1.64 (m, 5H), 1.70–1.93 (m, 3H), 2.23–2.48 (m, 4H), 2.52 (t, 2H, J = 6.0 Mhz) 2.54 (t, 2H, J = 6.0 Mhz), 2.66 (m, 1H), 4.68 (m, 1H), 7.09 (m, 1H), 7.18 (m, 1H), 7.36 (m, 1H), 7.42 (m, 2H), 9.40 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C; 12.08 (**C**-25), 12.10 (**C**–26), 20.60 (**C**-16), 23.83 (**C**–20), 24.05 (**C**–24), 27.60 (**C**–21), 29.20 (**C**-12), 29.48 (**C**-30, **C**–31), 31.96 (**C**–13), 33.68 (**C**–9), 35.60 (**C**–14), 37.03 (**C**–17), 37.79 (**C**–10), 40.55 (**C**–11), 43.93 (**C**–18), 51.44 (**C**–19), 54.76 (**C**–15), 79.80 (**C**–22), 105.72 (**C**–3), 110.56 (**C**–8), 117.63 (**C**–6), 118.21 (**C**–5), 120.79 (**C**–4), 126.65 (**C**–7), 133.72 (**C**–23), 135.33 (**C**–2), 171.80 (**C**–32), 173.60 (**C**–28) ppm. EI-MS *m/z:* 463.20 (M^+ 13). Anal. Calcd. for $C_{29}H_{37}NO_4$: C, 75.13; H, 8.04, N, 3.02; O, 13.80. Found: C, 75.10; H, 8.00

Method B

The compound **3** (100 mg, 0.27 mmol) was added to a solution of 65 mg succinic acid (0.55 mmol) and 100 mg 1,3-dicyclohexylcarbodiimide (0.48 mmol) in 15 cm3 acetonitrile-water (3:1) and 69 mg *p*-toluenesulfonic acid monohydrate (0.36 mmol) was added and the mixture was stirred at room temperature for 72 h. The solvent was then removed under vacuum and the crude product was purified by crystallization from methanol-hexane-water (3:2:1) yielding 78% of product. Similar ^1H NMR and ^{13}C NMR data were obtained compared with method A product.

RESULTS AND DISCUSSION

In this study, an indol-dihydrotestosterone succinate derivative (**4**) was synthetized; the first stage involves the synthesis of an indol-dihydrotestosterone derivative (**3**). It is important to mention that several protocols have been development for preparation of indol derivatives, nevertheless different protocols suffers from several drawbacks; 1) The products of reaction have limited stability and 2) the need to use hazardous reagents for their preparation[8,12]. In this study, the compound **3** was synthetized by reacting dihydrotestosterone (**1**) with phenylhydrazine (**2**) in presence of hydrochloric acid to form 3-indole-dihydrotestosterone derivative; (Figure 1, see). ^1H NMR spectra of **3** showed chemical shifts at 0.76 and 0.78 ppm for methyls present in the steroid nucleus. In addition, other signals at 0.98-4.01 ppm for hydrogens involved in the steroid nucleus were found. Other signals at 7.09-7.42 ppm for methylenes involved in the phenyl group were display. Finally, a signal at 7.49 ppm for protons involved in both amine and hydroxyl groups were found. The ^{13}C NMR spectra display chemical shifts at 11.12 and 12.40 ppm for the carbons of methyls presents in the steroid nucleus of **3**. Another chemical shifts at 20.60-76.86 ppm for carbons of methylenes involved in the steroid nucleus were exhibited. Finally, several signals at 105.72-135.33 ppm for carbons corresponding to methylenes involved in the indol group were found. The presence of **3** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 363.16.

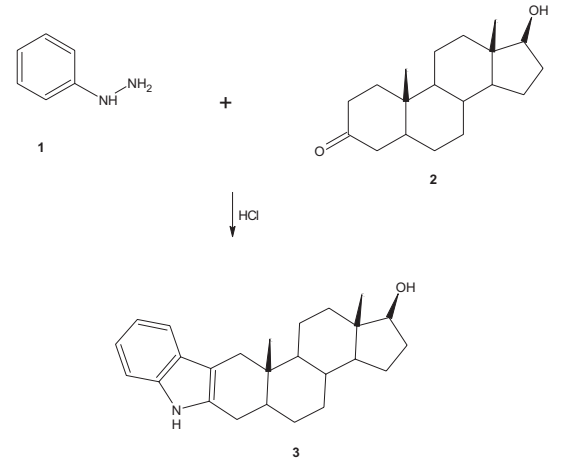

Fig. 1. Synthesis of an indol-dihydrotestosterone derivative (**3**). Reaction of dihydrotestosterone (**2**) with phenylhydrazine (**1**) using hydrochloric acid as catalyst to form of **3**.

On the other hand, the second stage was achieved by the synthesis of **4** which contains in the D-ring of the steroid nucleus an arm with ester functional group coupled to the steroid nucleus of **4**. It is important to mention, that there are diverse reagents to produce esters derivatives, nevertheless; most of the conventional methods have found only a limited use for this purpose[13,14]. In this study, two different methods were used; in the first step the technique reported by Figueroa and coworkers[15] for esterification of steroids using the compound **3**, succinic anhydride and pyridine (method A) for ester bond formation en the new arm bound to nucleus steroid in the compound **4** (Figure 2, see)

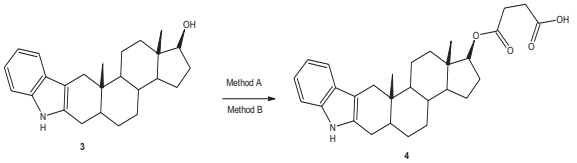

Fig. 2. Synthesis of an indol-dihydrotestosterone succinate derivative (**4**). Method A. Reaction of **3** with anhydride succinic using as catalyst pyridine to form **4**; Method B. Reaction of **3** with succinic anhydride using as catalyst 1,3-dicyclohexylcarbodiimide in presence of *p*-toluenesulfonic acid monohydrate to form **4**.

was used; in the second step was achieved by the reaction of **3** with succinic acid in presence of 1,3-dicyclohexylcarbodiimide to form **4** (method B). It is important to mention that during recent years, some carbodiimides derivatives such as the dicyclohexylcarbodiimide (DCC) have attracted increasing attention as condensing agents in ester synthesis[16,17]. Nevertheless, it is important to mention that when dicyclohexylcarbodiimide is used as condensing agent in esters synthesis, yields of the esters are often unsatisfactory because of formation of the N-acylurea derivative as by-product. Some reports reveal that addition of a catalytic amount of a strong acid to the esterification reaction in the presence of dicyclohexylcarbodiimide considerably increases the yield of esters and reduces the formation of the N-acylurea compound[18]. For this reason, esterification of the hydroxyl group of **3** with succinic acid in the presence of dicyclohexylcarbodiimide and *p*-toluenesulfonic acid (Scheme 2) was used to increase the yield of **4**. It was found that the use of carbodiimide derivative results in higher yields compared to the ester bond formed with method A.

The results indicate that ^{1}H NMR spectrum of **4** showed signals at 0.78 and 0.80 ppm for methyls present in the steroid nucleus. Additionally, other signals at 1.01–2.48, 2.66 and 4.68 ppm for hydrogens involved in the steroid nucleus were found. Other signals at 2.52–2.54 ppm for methylenes involved in arm bound to steroid nucleus of **4**; at 7.09–7.42 ppm for phenyl group were display. Finally, a signal at 9.40 ppm for both amine and hydroxyl groups were found. The ^{13}C NMR spectra display chemical shifts at 12.08 and 12.10 ppm for the carbons of methyls presents in the steroid nucleus of **4**. Other chemical shifts at 20.60–29.20 and 31.96–79.80 ppm for carbons of methylenes involved steroid nucleus; at 29.48 ppm for methylenes involved in arm bound to steroid nucleus were exhibited. Finally, other signals at 105.72–135.33 ppm for indol group; at 171.80 ppm for carboxyl group and at 173.80 ppm for ester group were exhibited. The presence of **4** was further confirmed from mass spectrum which showed a molecular ion at *m/z* 463.20.

CONCLUSIONS

In this work, we report an easy methodology to synthesize indol-dihydrotestosterone succinate derivative.

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ЛЕСНА СИНТЕЗА НА ПРОИЗВОДНО НА ИНДОЛ-ДИХИДРОТЕСТОСТЕРОН СУКЦИНАТА

Л. Фигероа-Валверде[1], Ф. Диас-Седильо[2], Е.Гарсиа-Сервера[1]

[1]*Лаборатория по фармацевтична химия, Факултет по химико-биологични науки, Автономен университет на Кампече, Мексико*
[2]*Висше училище по биологични науки, Национален политехнически институт, Санто Томас, Мексико*

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Синтезирано е производно на индол-дихидротестостерон сукцината (**4**). В първия етап, свързан с приготвянето на производното на индол-дихидротестостерона (**3**) чрез реакцията с дихидротестостерон (**1**) с фенилхидразин с хлороводород като катализатор. Вторият етап се извършва, като **3** реагира с янтарен анхидрид или с янтарна киселина и се получава производното на индол-дихидротестостерон сукцината (**4**).