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Synthesis of Pregnenolone-Pregnenolone Dimer via Ring A-Ring A connection

Lauro Figueroa Valverde,*[1] Francisco Díaz Cedillo,[2] Ligia Tolosa,[1] Guadalupe Maldonado,[1] Guillermo Ceballos Reyes[3]

[1] Universidad Autónoma de Campeche, Av. Agustín Melgar, Col Buenavista C.P.24030 Campeche Campeche, México., Telephone: (981)8184246, (981) 8119800 Ext. 73006, e-mail: lauro_1999@yahoo.com
[2] Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Col. Santo Tomás, México, D.F., C.P. 11340.
[3] Escuela Superior de Medicina, Instituto Politécnico Nacional, Díaz Mirón esq. Plan de Ayala s/n Col. Plutarco Elías Calles, México, D.F., C.P. 11340.

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Abstract. A pregnenolone-pregnenolone dimer was synthesized. The route involved preparation of pregnenolone hemisuccinate (**2)** by the esterification of pregnenolone (**1)** using succinic anhydride and pyridine, followed by formation of pregnenolone-derivate (**3)** and preparation of pregnenolone-pregnenolone dimer (**4)**.
Keywords: Pregnenolone, dimer, hemisuccinate.

Resumen. En este trabajo, fue sintetizado un dímero pregnenolona-pregnenolona. La ruta incluye la preparación del hemisuccinato de pregnenolona (**2)** por la esterificación de pregnenolona (**1)** usando anhídrido succínico y piridina, seguido por la formación del derivado de pregnenolona (**3)** y preparación del dímero pregnenolona-pregnenolona (**4**).
Palabras clave: Pregnenolona, dímero, hemisuccinato.

Introduction

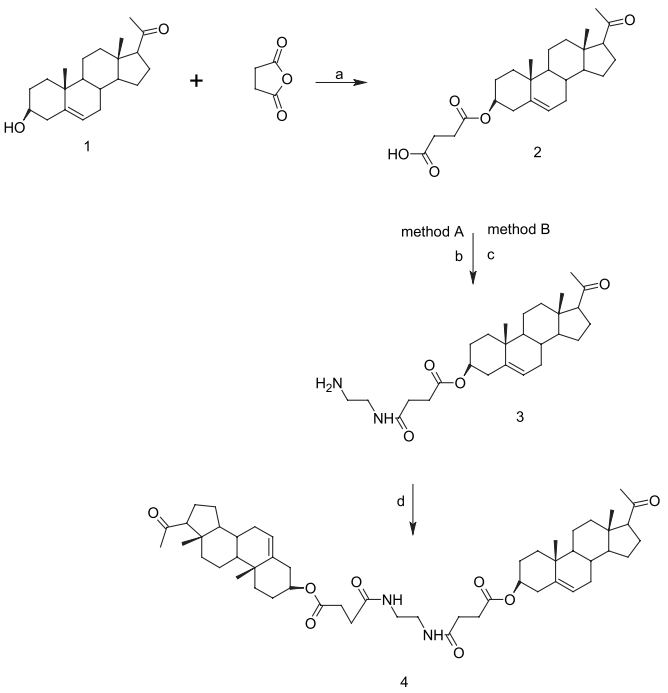
In the nervous system, neurosteroids are synthesized *de novo* or they are metabolic products of steroids derived from a peripheral source [1,2]. Their occurrence in the central and peripheral nervous system is independent, at least partially, of the endocrine secretion of steroids [3]. It has been demonstrated that neurosteroids modulate neurotransmission by binding to specific receptors and exert physiological functions that are clearly different from those of endocrine steroids [4]. Known neurosteroids include pregnenolone (3β-hydroxypregn-5β-en-20-one) and its reduced metabolite, pregnenolone sulfate, among others [5]. Pregnenolone is involved in several pathophysiological events, such as response to stress, depression, anxiety, sleep, epilepsy, and memory formation [6]. In this sense, pregnenolone is a potent neuromodulator that is formed by oxidative side-chain cleavage reaction from cholesterol [7]. Its biological activity is dependent on structural features of the steroid A and D-rings. In this context, several investigators have prepared a number of compounds in order to verify the hypothesis that specific a conformation of the functional groups is required for high biological activity, as example, Jiang and co-workers [8] synthesized several analogs of 3β-hydroxy-5β-pregnan-20-one and evaluated its activity in electrophysiological experiments using rat R1,2Á2L $GABA_A$ receptors expressed in *Xenopus laevis* oocytes. In addition, other studies have shown the synthesis of 6-oxa-analogs of neurosteroids and $GABA_A$ receptor activity [9].

On the other hand, steroids dimers exist in the natural kingdom and the study of their synthesis and effects are emerging in different areas of physiology and pharmacology. In this regard there are reports showing that a pregnenolone-pregnenolone dimer via Ring A-Ring A connection through spacer groups can be obtained by using appropriate functional groups. As example, Neder and co-workers [10] synthesized 3,3'-ether-3β-hydroxypregn-5-en-20-one dimmer that was obtained from the reaction mixture of 3-hydroxypregn-5-en-20-one and 2-chloro-*N*,*N*-diethyl-1,1,2-trifluoroethanamine compound. Other reports [11] showed the synthesis of a pregnenolone-dimer via Ring D-Ring D connection and a 1,3-disubstituted imidazolium adduct.

Here we report a method that involves the modification of the hydroxyl (C-3 A-ring) group of pregnenolone, in order to develop new strategies to synthesize a pregnenolone dimer with and without spacer groups that can be used as chiral building blocks to construct artificial receptors and as architectural components in biomimetic/molecular recognition chemistry.

Results and discussion

In this work we report several straightforward routes for the synthesis of dimeric pregnenolone (see Scheme 1). The first step involves the esterification of the hydroxyl group (C-3 A-ring) of pregnenolone to form the pregnenolone hemisuccinate (5-Pregnen-20-one, 3-(3-carboxy-1-oxopropoxy) by a modification of the methods reported by Yellin [12], and Bernês [13] for the synthesis of hemisuccinate-steroids, using toluene to avoid hydrolysis in the new arm formed in A-ring of the pregnenolone-derivate, that has both characteristic ester and carboxyl groups. The results indicate that the ^{1}H NMR spectrum of pregnenolone hemisuccinate showed a signal at δ 10.03 corresponding to the C(=O)-OH. The presence of the preg-



Scheme 1. Synthesis of pregnenolone-pregnenolone dimer via Ring A-Ring A connection; a) pyridine/toluene; b) ethylenediamine dihydrochloride, boric acid/toluene; c) ethylenediamine dihydrochloride, acetonitrile/water; d) EDC, acetonitrile/water

nenolone hemisuccinate was further confirmed from mass spectrum which showed a molecular ion at m/z 416.

The second step was achieved by reacting ethylenediamine with pregnenolone hemisuccinate resulting in amide bond formation. It is important to mention that many procedures for the formation of amide groups are known in the literature, the most widely practiced method employs carboxylic acid chlorides as the electrophiles which react with the amine in the presence of an acid scavenger [14]. Despite its wide scope, this protocol suffers from several drawbacks; most notable are the limited stability of many acid chlorides and the need for hazardous reagents for their preparation (thionyl chloride) [15]. In this work two different methods for amide formation were employed, in the first one the technique reported by Pingwah [16] for boric acid catalyzed amidation of carboxylic acids and amines (method A) was used, in the second one we used a derivate of carbodiimide (method B) as catalyzer [17] for amide bond formation in the new arm formed in A-ring of pregnenolone. It was found that the use of carbodiimide results in higher yields compared to the amide bond formed with method A.

The presence of the -O-C=O-NH-$(CH)_2$-NH_2 fragment was confirmed by ^{1}H NMR which showed a signal for the -C-NH_2 fragment at 1.2 (CH_2N) and one at 3.72 (CO_2-NH). It is important to mention that the ^{1}H NMR spectra of the secondary amides are usually more complex than the primary amides due to the presence of a substituent bonded to the amide nitrogen atom. These substituents produce a much wider range of chemical shifts for the amide proton which may, in addition, display coupling to aliphatic groups bonded to it. The chemical shifts of aliphatic groups bonded to the carbonyl group are similar to those observed for the primary amides, while those groups bonded to the-nitrogen resonate at slightly lower field than the corresponding amines. In addition, the presence of the pregnenolone-derivate (**3**) was further confirmed from the mass spectrum which showed a molecular ion at m/z 458.

On the other hand, the synthesis of the pregnenolone-dimer (**4**) via Ring A-Ring A connection, was obtained binding pregnenolone hemisuccinate (**2**) to pregnenolone-derivate (**3**) using a carbodiimide-derivate for the formation of the new amide group in the pregnenolone-dimer. The presence of the -O-C=O-$(CH)_2$-C=O(O)-NH-$(CH)_2$-NH-(O)O=C-$(CH)_2$-O=C-O- fragment was confirmed by ^{1}H NMR that showed a signal proton at 3.71 (CO_2-NH) whereas the signal of amine group is absent. In addition, the mass spectrum of this dimeric molecule exhibited a molecular ion at m/z 856 (M^+) that confirmed the structure.

Conclusions

We report an easy methodology to synthesize pregnenolone-dimer via Ring A-Ring A connection. The purpose to use of long spacer arm in the pregnenolone-dimer is to avoid the steric hindrance that the support could have when interacting with other biological molecules. This dimer has enough flexibility and it allows free movement in comparison with other dimers synthesized via ring A-ring A, for example the 3,3′-ether-3-hydroxypregn-5-en-20-one that has a short spacer arm [10,11].

Experimental

General methods

Melting points were determined on an Electrothermal (900 model). Ultraviolet spectroscopy (UV) was carried out in dry methanol on a Perkin-Elmer model 552 spectrophotometer and infrared spectra (IR) was 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 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GC-Polaris Q. spectrometer. Elemental analyses were obtained at the Centro de Investigaciones Químicas, Universidad Autónoma de Hidalgo, México.

Synthesis of pregnenolone hemisuccinate (5-Pregnen-20-one, 3-(3-carboxy-1-oxopropoxy) (1) . A solution of pregnenolone (3β-hydroxypregn-5β-en-20-one) 200 mg (0.95mmol), succinic anhydride 142 mg (1.42 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) to give 235 mg (80 %), mp 165 °C; UV (MeOH) λ_{max} (log) 215 (2.74) nm; IR V_{max} 3505, 1700 cm^{-1}; ^1H NMR (75 MHz, $CDCl_3$) δ_H: 0.62 (3H, s H_3-18), 0.92-1.14 (1H, m), 1.01(3H, s, H_3-19), 1.12-1.35 (4H, m), 1.45-1.57 (3H, m), 1.58-1.73 (4H, m), 1.93-2.19 (2H, m), 2.06, (3H, s, H_3-21), 2.35 (5H, m), 3.42 (1H, m, H-3), 5.33 (1H, d, J = 4.5 Hz, H-6), 10.03 (1H, br, CO_2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 13.40 (C-18), 19.52 (C-19), 21.92 (C-11), 22.90 (C-15), 23.61 (C-21), 27.80 (C-2), 29.45 (RO_2C-C-C-CO_2H), 31.40 (C-16), 31.80 (C-8), 32.6 (C-7), 36.74 (C-10), 37.01 (C-1), 38.29 (C-4), 38.80 (C-12), 43.90 (C-13), 49.94 (C-9), 56.78 (C-14), 63.60 (C-17), 73.95 (C-3), 122.69 (C-6), 139.60 (C-5), 173.85 (CO_2R), 177.30 (CO_2H); EIMS(30 ev) *m/z* (rel. int.), 416 (12, M^+), 298 (64), 283 (30), 255 (27), 213 (58), 209 (27), 161 (28), 147 (46), 105 (66), 91 (100). Anal. Calcd. for C_{25} H_{36} O_5: C, 72.08; H, 871; O, 19.20. Found: C, 72.01; H, 9.02.

***N*-(2-amino-ethyl)-succinamic acid 17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12, 13,14,15,16,17-tetradecahydro-1H-cyclopenta[á]phenanthren-3-yl-ester (3).**

Method A. A solution of 82 mg ethylenediamine dihydrochloride (0.62 mmol), 26 mg boric acid (0.42 mmol) and 200 mg of pregnenolone hemisuccinate (0.41 mmol) in toluene (10 mL) was heated under reflux for 5 h. The reaction was allowed to cool to room temperature and the toluene was evaporated under vacuum. The reaction mixture was dissolved in ethyl acetate (10 mL) and water (20 mL); the organic layer was separated, and the aqueous layer was re-extracted with ethyl acetate (10 mL). The combined organic layers were washed with water (15 mL), and then dried over sodium carbonate (anhydrous). The organic phase was evaporated to dryness under reduced pressure. The residue was purified by crystallization from methanol:water (3:1) to give 120 mg (22%), mp 212-213 °C; UV (MeOH) λ_{max} (log ε) 218 (2.32) nm; IR V_{max} 3360, 1685 cm^{-1}; ^1H NMR (75 MHz, $CDCl_3$) δ_H: 0.64 (3H, s H_3-18), 1.02(3H, s, H_3-19), 1.08-1.35 (4H, m), 1.39-1.68 (8H, m) 1.82- 2.09 (3H, m), 2.11- 2.21 (1H, m),2.13, (3H, s, H_3-21), 2.30-2.39 (2H, m), 2.55-2.71 (5H, m), 3.63 (4H, m), 4.17 (1H, br), 4.62(1H, m, H-3), 5.39 (1H, d, J = 4.8 Hz, H-6); 3.72 (1H, CO_2NH), 1.2 (1H, C-NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 13.17 (C-18), 19.53 (C-19), 21.90 (C-11), 22.90 (C-15), 23.63 (C-21), 27.79 (C-2), 29.42 (RO_2C-C), 30.43(-C-CO_2NH), 31.43 (C-16), 31.80 (C-8), 32.6 (C-7), 36.74 (C-10), 37.01 (C-1), 38.29 (C-4), 38.80 (C-12),41.99 (C-NH), 42.68(C-NH_2), 43.93 (C-13), 49.94 (C-9), 56.70 (C-14), 63.63 (C-17), 73.95 (C-3), 122.68 (C-6), 139.60 (C-5), 173.85 (CO_2R), 177.33 (CO_2NH). EIMS(30 ev) *m/z* (rel. int.) 458 (M^+,3), 316(20), 314(16), 239(100), 223 (30), 91 (100), 70.7 (60). Anal. Calcd for $C_{27}H_{42}N_2O_4$: C, 70.71; H, 9.23; N, 6.11; O, 13.95. Found: C, 71.63; H, 9.29; N, 5.98.

Method B. Pregnenolone hemisuccinate 200 mg (0.41 mmol) was added to a solution of ethylenediamine dihydrochloride (82 mg, 0.62 mmol) and 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide hydrochloride (EDC) in acetonitrile-water (10 ml, 4:1). After stirring at room temperature for 28 h, the solution was concentrated and the product was extracted with ethyl acetate and water (2:1) to give pregnenolone-derivate (**3)** (180 mg, 68% yield) similar ^1H NMR and ^{13}C NMR data were obtained compared with method A product.

N-2-[3-(17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,-13,14,15,16,17-tradecahydro- 1H-cyclopenta[α]phenanthren-3-yloxycarbonyl)-propionylamino]-ethyl}-succinamic acid 17-acetyl-10,13-dimethyl-2,3,4,7,8,9,10,11,12,-13,14,15,16,17-tetradecahy dro-1H-cyclopenta[α]phenanthren-3-yl ester (**4)**. The pregnenolone-derivate (**3**) (303 mg, 0.72 mmol) was added to a solution of pregnenolone hemisuccinate (200 mg, 0.48 mmol) and 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide hydrochloride (EDC) in acetonitrile-water (10 mL, 2:1). The mixture was stirred at room temperature for 36 h, the solvent was removed under vacuum and the crude product was purified by crystallization from methanol:water (3:1) to give 220 mg (55%), mp 155-156°C. UV (MeOH) λ_{max} (log e) 211 (2.54) nm; IR V_{max} 3447, 1685 cm^{-1}; ^1H NMR (75 MHz, $CDCl_3$) δ_H: 0.64 (6H, s H_3-18), 1.01(6H, s, H_3-19), 1.11-1.13 (3H, m), 1.42-1.75 (8H, m),1.81-1.96 (3H, M), 1.95-2.01 (3H, m), 2.18, (6H, s, H_3-21), 2.19-2.25 (2H, m), 2.31-2.42 (2H, m), 2.61-2.79 (2.55 (8H, m) 3.71 (4H, s, CO_2NH), 4.65 (2H, m, H-3), 5.39 (2H, d, J = 4.8 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 13.19 (C-18), 13.24 (C'-18), 19.26 (C'-19), 19.31 (C-19), 21.02 (C-11), 21.82 (C'-11), 24.47 (C-21), 27.64 (C-2), 28.87 (RO_2C-C), 29.22 (RO_2C-C), 31.52 (-C-CONH), 31.57 (-C-CONH), 31.75 (C-16), 31.80 (C'-16), 31.99 (C-7), 36.58 (C-1), 36.94 (C'-1), 37.95 (C-10), 38.77 (C-4), 43.99 (CNH), 45.46 (C-12), 49.85 (C-13), 50.02 (C-9), 56.82 (C-14), 63.68 (C-17), 74.33 (C-3), 74.44 (C'-3), 122.37 (C-6), 122.47 (C'-6), 139.53 (C-5), 171.55 (CO_2R), 177.16 (CONH), 209.70 (C-20);EIMS (30ev) m/z (rel. int.) 856 (M^+, 9), 332 (10), 316 (61), 239 (100), 197 (65), 183(52), 91 (68), 70 (92). Anal. Calcd for $C_{52}H_{76}N_2O_8$: C, 72.86; H, 8.94; N, 3.27; O, 14.93 Found: C, 72.73; H, 9.11; N, 3.58.

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