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CONVENIENT SYNTHESIS OF NEW PREGNENOLONE OXIMINYL OXALATE DIMERS

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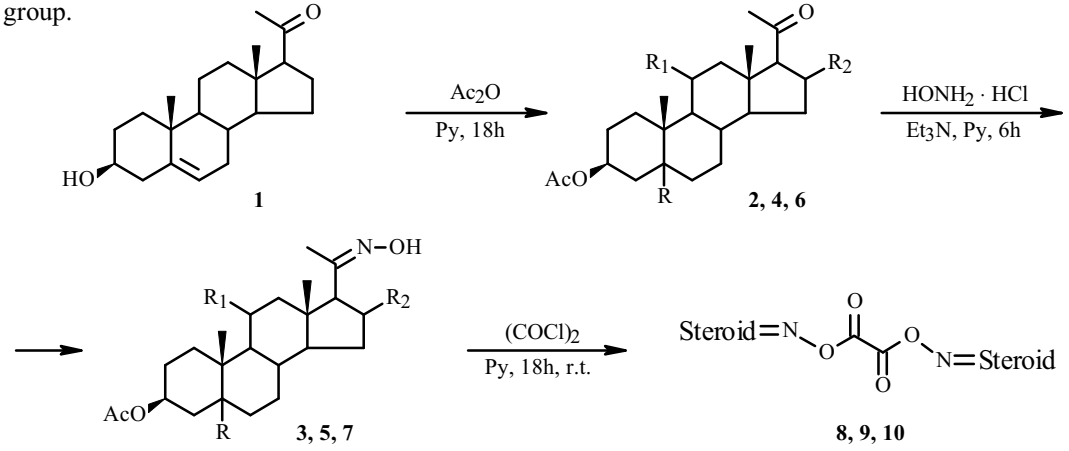
*Three new symmetrical pregnenolone oxyminyl oxalate dimers (**8-10**) were synthesized from the corresponding pregnenolone oximes (**3**, **5**, and 7) at room temperature. All dimers were characterised by spectroscopic means, notably HRFABMS and comprehensive NMR spectroscopic data analyses.*

Key words: pregnenolone, oxime, esterification, oxalate dimers, NMR.

In continuation of the search for biologically active steroid-based dimers [1], we have used pregnenolone (**1**) as the starting substance. Pregnenolone, a well-known vertebrate hormone, is a primary steroid on the pathway from cholesterol to the androgens, estrogens, progesterone, and the corticoids, and also the starting material for several other pharmacologically active steroids [2, 3]. As part of our initiative for synthesizing new steroid dimers towards the generation of a library of steroidal dimers for relevant pharmacological screening, we now report on the synthesis of three new pregnenolone oximinyl oxalate dimers (**8**-**10**), which contain an oxalate ester linkage between the 20-one oxime positions of two molecules.

Oximes, as they contain a hydroxyl group linked to nitrogen, behave like alcohols, and are considered to be important reaction intermediates for the synthesis of various nitrogen-containing compounds. To synthesize the target dimer bis(3β-acetoxypregn-5-en)-20-on-oximinyl oxalate (**8**), it was necessary to prepare the steroid monomer 3β-acetoxypregn-5-en-20-one oxime (**3**). In order to functionalize C-20 of pregnenolone (**1**), its 3β-hydroxy group was protected by acetylation [4-6]. The acetylated pregnenolone **2** was treated with hydroxylamine hydrochloride to obtain 20-one oxime **3** [4]. An increase of 15 mass units in its molecular mass (compared to that of **2**) obtained from the ESIMS spectrum ($[M+H]^+$ and $[M+Na]^+$ at *m/z* 374 and 396, respectively) confirmed the presence of a C=N-OH instead of a C=O group. The ^{1}H NMR showed a broad 1H singlet at δ 8.88 for hydroxyl proton, and an olefinic quaternary carbon at δ 158.7 observed in its ^{13}C NMR spectrum was characteristic for C=N-OH group.



2, 3: R = 5-dehydro, $R_1 = R_2 = H$; **4, 5:** R = 5-dehydro, $R_1 = H$, R_2 = 16-dehydro

6, 7: 5α-H, R_1 = 11α-OAc, R_2 = 16-dehydro

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TABLE 1. ^{13}C NMR ($CDCl_3$, 100 MHz) Data of Dimers **8-10**

C atom	Chemical shifts (δ, ppm)		
	8	**9**	**10**
1, 1′	36.8	36.9	37.1
2, 2′	27.8	27.7	27.7
3, 3′	73.8	73.8	73.1
4, 4′	38.1	34.6	34.4
5, 5′	139.7	140.2	44.6
6, 6′	122.2	122.0	28.8
7, 7′	31.7	32.1	33.2
8, 8′	32.0	30.2	33.5
9, 9′	50.0	50.2	55.5
10, 10′	36.6	36.7	36.7
11, 11′	21.0	20.9	70.5
12, 12′	38.4	38.1	42.2
13, 13′	44.0	46.5	46.3
14, 14′	56.2	56.7	56.9
15, 15′	24.1	31.4	31.9
16, 16′	23.4	139.5	139.2
17, 17′	56.6	149.7	149.0
18, 18′	13.2	13.4	12.7
19, 19′	19.3	15.4	13.4
20, 20′	167.5	159.5	159.0
21, 21′	17.1	19.2	16.7
2×CO (oxalate)	170.5	170.5	170.5
2×Me (3β-OAc)	21.4	21.4	21.4
2×CO (3β-OAc)	170.5	170.5	170.5
2×Me (11α-OAc)	-	-	21.9
2×CO (11α-OAc)	-	-	170.7

Similarly, two other oximes, 3β-acetoxypregna-5,16-dien-20-one oxime (**5**) and 3β,11α-diacetoxy-5α-pregn-16-en-20-one oxime (**7**) were synthesised, respectively, from acetylated ketonic derivatives **4** and **6** as the precursors for corresponding oximinyl oxalate dimers (**9** and **10**). The identity of **5** was confirmed by comparison of its mp, IR, MS, and ^1H NMR data with published data [7]. The ESIMS spectrum of **5** revealed the $[M+H]^+$ and $[M+Na]^+$ ions, respectively, at *m/z* 372 and 394, which confirmed the molecular formula, $C_{23}H_{33}NO_3$, for this compound. An olefinic quaternary carbon at δ 154.5 in its ^{13}C NMR spectrum was characteristic for the C=N-OH group.

The oxime **7** was synthesized previously as a reaction intermediate and identified by forming 3β,11α-diacetoxy-5α-androstan-17-one [8], but no physical or spectroscopic data for this compound could be found in the literature. The HRFABMS spectrum of **7** revealed the $[M+H]^+$ ion at *m/z* 432.274978 which confirmed the molecular formula, $C_{25}H_{37}NO_5$, for this compound. This fact was confirmed further from a broad 1H singlet at δ 7.51 (for the hydroxyl proton) in its ^1H NMR and a signal for an olefinic quaternary carbon at δ 153.3 for C=N-OH in its ^{13}C NMR spectrum.

Bis(3β-acetoxypregn-5-en)-20-on-oximinyl oxalate (**8**) was synthesized from the reaction between 3β-acetoxypregn-5-en-20-one oxime (**3**) and oxalyl chloride. The HRFABMS spectrum of **8** exhibited the $[M+H]^+$ ion at *m/z* 801.50536 corresponding to $C_{48}H_{69}N_2O_8$ and thus confirmed the formation of the dimer. In the ^{13}C NMR spectrum (Table 1), the chemical shifts of four carbonyl carbons, two of the oxalates and two of the acetate moieties, appeared at δ 170.5. The oxalate carbonyls contributed to the downfield shift of C-20 carbon that appeared at δ 167.5 as opposed to δ 158.7 for the parent oxime.

Conjugated oximes show enhanced reactivity in biological environment compared to free oximes [8]. The steroid monomer, 3β-acetoxypregna-5,16-dien-20-one oxime (**5**), is an important key intermediate for the synthesis of antitumor drugs [9, 10]. It has recently been found that 3β-acetoxypregna-5,16-dien-20-one oxime (**5**) is a potent inhibitor of human $P450^{17}\alpha$ [7]. Therefore, synthesis of the dimer of **5** via oxalic acid spacer appeared to be an interesting prospect as the dimer may have the potential of exerting enhanced antitumor properties compared to its monomer. Bis (3β-acetoxypregna-5,16-dien)-20-on-oximinyl oxalate (**9**) was synthesized from 3β-acetoxypregna-5,16-dien-20-one oxime (**5**) using oxalyl chloride. The molecular

mass of **9** was confirmed from its HRFABMS spectrum where the $[M+H]^+$ was observed at m/z 797.47406 corresponding to $C_{48}H_{65}N_2O_8$.

Another conjugated 16-dehydro oxime dimer was synthesized in a similar manner. Bis (3β,11α-diacetoxy-5α-pregn-16-en)-20-on-oximinyl oxalate (**10**) was synthesised from 3β,11α-diacetoxy-5α-pregn-16-en-20-one oxime (**7**) using oxalyl chloride. In its HRFABMS spectrum, the $[M+H]^+$ ion at *m/z* 917.51633 corresponding to $C_{52}H_{73}N_2O_{12}$ confirmed the identity of this dimer. The ^{13}C NMR spectral behavior of **9** and **10** (Table 1) was similar to that observed for the dimer **8**, especially in relation to the chemical shifts of the carbonyl carbons and C-20 olefinic quaternary carbon. In both cases, all four carbonyl carbons gave a single resonance at δ 170.5, and the C-20 carbon signal was deshielded compared to those of their parent monomers.

EXPERIMENTAL

General Procedures [1]. Pregnenolone (**1**), 3β-hydroxypregna-5,16-dien-20-one, 3β,11α-dihydroxy-5α-pregn-16-en-20-one oxalyl chloride, and dry pyridine were purchased from Aldrich and used as received.

3β-Acetoxypregn-5-en-20-one (2). A stirred solution of pregnenolone (**1**, 1.0 g, 3.16 mmol) in dry pyridine (5 mL) was added to Ac_2O (3 mL) and stirred for 18 h at room temperature. Then the reaction mixture was quenched with ice-H_2O, and a white precipitate was formed. The precipitate was collected, taken into ether, dried over $MgSO_4$, and evaporated to afford the title compound **2** (990 mg, 87%) as a white solid, mp: 136-137°C (lit. mp: 135-137°C [4], IR [5], MS [5], ^1H NMR and ^{13}C NMR [6]). A similar synthetic procedure was followed for the synthesis of compounds **4** and **6**.

3β-Acetoxypregn-5-en-20-one Oxime (3). A stirred solution of (**2**, 930 mg, 2.59 mmol) in dry pyridine (5 mL) was treated with hydroxylamine hydrochloride (360 mg, 5.19 mmol) and Et_3N (5 mL). The mixture was refluxed for 6 h. Finally, the reaction mixture was quenched with ice-H_2O, and a white precipitate was formed. The precipitate was collected and taken into EtOAc, dried over $MgSO_4$, and evaporated to afford the title compound **3** (851 mg, 88%) as a white solid, mp: 183-185°C (lit. mp: 187-189°C [4]). IR ($CHCl_3$, ν_{max} cm^{-1}): 3313br (O-H), 2937s (C-H), 2854s (C-H), 1732vs (ester C=O), 1654m (C=C), 1439m, 1369m, 1249s (ester C-O), 1041m and 668m. ^1H NMR (400 MHz, $CDCl_3$, δ): 0.63 (s, 3H, 18-Me), 1.00 (s, 3H, 19-Me), 2.01 (s, 3H, 21-Me), 1.99 (s, 3H, 3β-OCO-Me), 4.57 (m, 1H, 3α-OCH), 5.36 (br m, 1H, 6-CH), 8.88 (br s, 1H, =N-OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.0 (C′-1), 27.8 (C-2), 73.9 (C-3), 38.1 (C-4), 139.7 (C-5), 122.4 (C-6), 31.8 (C-7), 32.0 (C-8), 50.1 (C-9), 36.6 (C-10), 21.0 (C-11), 38.6 (C-12), 43.8 (C-13), 56.8 (C-14), 24.2 (C-15), 23.1 (C-16), 56.1 (C-17), 13.1 (C-18), 15.2 (C-19), 158.7 (C-20), 19.3 (C-21), 170.6 (3E-OCO-Me), 21.5 (3E-OCO-Me). ESIMS: *m/z* 374 $[M+H]^+$, 396 $[M+Na]^+$.

3β-Acetoxypregna-5,16-dien-20-one Oxime (5). The title compound **5** (510 mg, 52%), mp 182-183°C (lit. mp 180-185°C [7], IR and ^1H NMR [7]) was synthesized from 3β-acetoxypregna-5,16-dien-20-one (**4**, 950 mg, 2.66 mmol) using the procedure described for compound **3**. ^{13}C NMR (100 MHz, $CDCl_3$, δ): 36.9 (C-1), 27.7 (C-2), 74.0 (C-3), 38.2 (C-4), 140.1 (C-5), 122.3 (C-6), 31.6 (C-7), 30.3 (C-8), 50.4 (C-9), 36.8 (C-10), 20.9 (C-11), 35.6 (C-12), 46.6 (C-13), 57.1 (C-14), 31.6 (C-15), 133.0 (C-16), 151.5 (C-17), 11.7 (C-18), 15.9 (C-19), 154.5 (C-20), 19.3 (C-21), 170.7 (3β-OCO-Me), 21.5 (3β-OCO-Me). ESIMS: *m/z* 372 $[M+H]^+$, 394 $[M+Na]^+$.

3β,11α-Diacetoxy-5α-pregn-16-en-20-one Oxime (7). The title compound **7** (500 mg, 48%), mp 120-122°C was synthesised from 3β,11α-diacetoxy-5α-pregn-16-en-20-one (**6**, 1.0 g, 2.40 mmol) using the procedure described for compound **3**. IR ($CHCl_3$, ν_{max} cm^{-1}): 3378 br (O-H), 2928s (C-H), 2857s (C-H), 1731s (ester C=O), 1590w (C=C), 1443m, 1372m, 1247s (ester C-O), 1021m, 962m and 753s. ^1H NMR (400 MHz, $CDCl_3$, δ): 0.89 (s, 3H, 18-Me), 0.91 (s, 3H, 19-Me), 1.99 (s, 3H, 21-Me), 1.91 (s, 3H, 11α-OCO-Me), 1.91 (s, 3H, 3β-OCO-Me), 4.61 (m, 1H, 3α-OCH), 5.17 (m, 1H, 11β-OCH), 5.96 (t, J = 2.1, 1H, 16-CH=C), 7.51 (br s, 1H, =N-OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 36.9 (C-1), 27.7 (C -2), 73.1 (C-3), 34.4 (C-4), 44.7 (C-5), 31.4 (C-6), 31.9 (C-7), 33.4 (C-8), 55.4 (C-9), 37.2 (C-10), 71.5 (C-11), 42.7 (C-12), 46.6 (C-13), 56.9 (C-14), 28.9 (C-15), 132.1 (C-16), 150.8 (C-17), 11.0 (C-18), 12.8 (C-19), 153.3 (C-20), 17.0 (C-21), 170.7 (11α-OCO-Me), 22.0 (11α-OCO-Me), 170.5 (3β-OCO-Me), 21.4 (3β-OCO-Me). FABMS: *m/z* 432 $[M+H]^+$, 454 $[M+Na]^+$. HRFABMS calc. for $C_{25}H_{38}NO_5$: 432.274978; found: 432.274978.

Bis(3β-acetoxypregn-5-en)-20-on-oximinyl Oxalate (8). A stirred solution of 20-one oxime **3** (200 mg, 0.54 mmol) in dry pyridine (5 mL) was treated with oxalyl chloride (34 mg, 0.27 mmol) in DCM. After 18 h, water (10 mL) was added slowly to the resulting mixture, precipitation occurred, and the precipitate was filtered off. It was then washed several times with

water to remove pyridine hydrochloride. Finally, the solid was dissolved in ether (10 mL) and evaporated to dryness at 35°C. Recrystallization from a mixture of (2:1) $CHCl_3$ and EtOAc yielded a white solid as the title compound **8** (125 mg, 58%), mp: 204-205°C. IR ($CHCl_3$, ν_{max} cm^{-1}): 2943s (C-H), 2853s (C-H), 1764s (oxime oxalate C=O), 1732s (oxime oxalate and acetate C=O), 1638m (C=C), 1438m, 1374m, 1247s (acetate C-O), 1134s (C-O), 1033m and 761m. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.64 (s, 6H, 18-Me and 18′-Me), 1.00 (s, 6H, 19-Me and 19′-Me), 2.02 (s, 6H, 21-Me and 21′-Me), 1.99 (s, 6H, 3-OCO-Me and 3′-OCO-Me), 4.57 (br m, 2H, 3-CH-O and 3′-CH-O), 5.35 (br m, 2H, 6-CH and 6′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 801 $[M+H]^+$, 823 $[M+Na]^+$. HRFABMS calc. for $C_{48}H_{69}N_2O_8$: 801.50536; found: 801.50536. Similar synthetic procedure was followed for the synthesis of dimers **9** and **10**.

Bis (3β-acetoxypregna-5,16-dien)-20-on-oximinyl Oxalate (9). The title compound **9** (150 mg, 47%, mp: 165-167°C) was synthesized using 20-one oxime **5** (300 mg, 0.81 mmol in dry pyridine (5 mL) and oxalyl chloride (51 mg, 0.40 mmol). IR ($CHCl_3$, ν_{max}, cm^{-1}): 3313 br (O-H) 2937s (C-H), 2854s (C-H), 1764s (oxime oxalate C=O), 1732s (oxime oxalate and acetate C=O), 1654m (C=C), 1439m, 1369m, 1249s (acetate C-O), 1132s (C-O), 1041m and 668m. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.83 (s, 6H, 18-Me and 18′-Me), 0.98 (s, 6H, 19-Me and 19′-Me), 2.09 (s, 6H, 21-Me and 21′-Me), 1.99 (s, 6H, 3-OCO-Me and 3′-OCO-Me), 4.56 (m, 2H, 3-CH-O and 3′-CH-O), 5.33 (br m, 2H, 6-CH and 6′-CH), 6.33 (br m, 2H, 16-CH and 16′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 797 $[M+H]^+$, 819 $[M+Na]^+$. HRFABMS calc. for $C_{48}H_{65}N_2O_8$: 797.47406; found: 797.47406.

Bis (3β,11α-diacetoxy-5α-pregn-16-en)-20-on-oximinyl Oxalate (10). The title compound **10** (424 mg, 38%, mp 229-230°C) was obtained from 20-one oxime **7** (1.0 g, 2.42 mmol) in dry pyridine (15 mL) and oxalyl chloride (154 mg, 1.21 mmol). IR ($CHCl_3$, ν_{max}, cm^{-1}): 3364 br (O-H) 2930s (C-H), 2860s (C-H), 1765s (oxime oxalate C=O), 1731s (oxime oxalate and acetate C=O), 1594w (C=C), 1449m, 1373m, 1258s (acetate C-O), 1131s (C-O), 1027m, 916m and 733s. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.91 (s, 6H, 18-Me and 18′-Me), 0.94 (s, 6H, 19-Me and 19′-Me), 1.89 (s, 6H, 11-OCO-Me and 11′-OCO-Me), 1.94 (s, 6H, 21-Me and 21′-Me), 1.91 (s, 6H, 3-OCO-Me and 3′-OCO-Me), 4.60 (m, 2H, 3-CH-O and 3′-CH-O), 5.18 (m, 2H, 11-CH-O and 11′-CH-O), 5.97 (t, J = 2.1, 2H, 16-CH and 16′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 917 $[M+H]^+$, 939 $[M+Na]^+$. HRFABMS calc. for $C_{52}H_{73}N_2O_{12}$: 917.51631; found: 917.51633.

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