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FACILE SYNTHESIS OF NEW OXALATE DIMERS OF NATURALLY OCCURRING 3-HYDROXYSTEROIDS

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*Several symmetrical steroidal oxalate dimers were synthesised from naturally occurring 3-hydroxysteroids, namely, estrone (**1**), DHEA (**3**), pregnenolone (**5**), cholesterol (**7**), 5D-cholestane (**9**), and stigmasterol (**11**), using oxalyl chloride. Full spectroscopic data for all the new compounds are presented.*

Key words: steroid, oxalyl chloride, esterification, oxalate dimers, NMR.

Steroid-based dimers were initially obtained by photocondensation and reductive coupling [1, 2], and are also found in nature [3, 4]. Later, steroid dimers were chemically synthesised via an ester and ether linkages [5–7]. It was also demonstrated that two cholic acid molecules or a cholic acid and a cholesterol molecule could form an ester linkage in the gastrointestinal and liver systems of mammals [5, 8]. Many dimeric steroids exhibit miceller, detergent, and liquid crystal behavior [9] and are pharmaceutically important [10]. They have been used as catalysts for certain organic reactions [11] and can be used to develop new pharmacologically active steroids [10]. In the search for new pharmacologically active steroids, one of the usual starting points could be the modification of naturally occurring steroid hormones and thus influence the biological activity. Bis-steroids obtained by dimerization of pharmacologically active steroids can be considered as such a modification [10]. 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 several new steroidal dimers containing an oxalate ester linkage between the C-3 positions of two molecules.

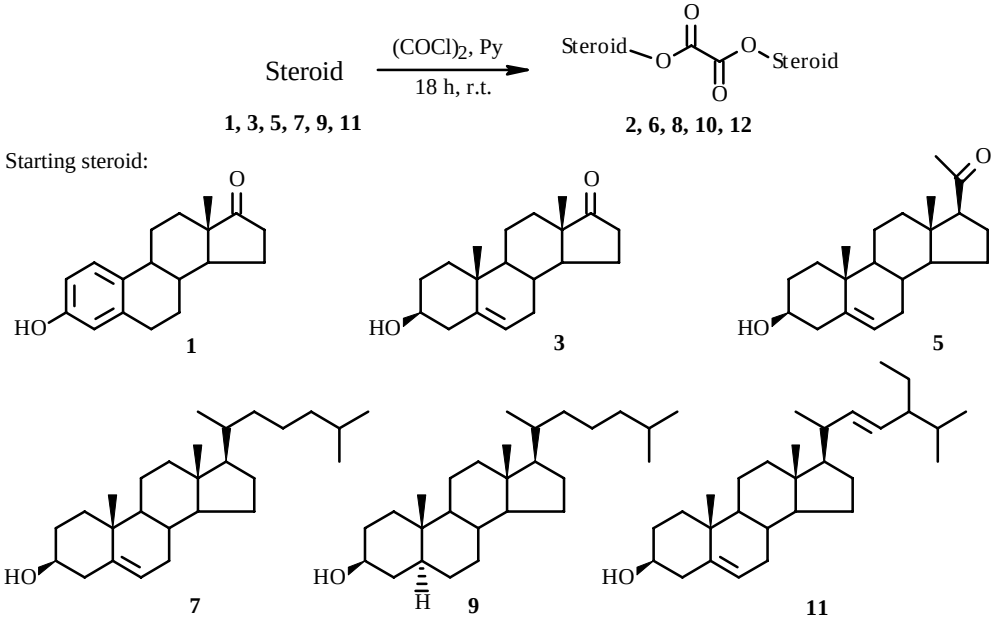

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TABLE 1. ^{13}C NMR ($CDCl_3$, 100 MHz) Data of Dimers **4, 6**, **8**, **10,** and **12**

Carbon No.	Chemical shifts (δ) in ppm				
	4	**6**	**8**	**10**	**12**
1 and 1′	36.8	36.8	36.9	36.7	36.9
2 and 2′	27.3	27.3	27.4	27.1	27.4
3 and 3′	76.9	77.1	77.2	77.0	77.2
4 and 4′	31.4	37.6	37.6	37.6	37.6
5 and 5′	139.3	139.0	139.0	44.7	139.0
6 and 6′	122.5	123.0	123.3	28.6	123.3
7 and 7′	30.8	31.8	31.9	31.9	31.9
8 and 8′	31.4	31.7	31.8	35.4	31.8
9 and 9′	51.7	49.8	50.0	54.2	51.2
10 and 10′	36.7	36.6	36.6	33.5	36.6
11 and 11′	20.3	21.0	21.0	21.2	21.0
12 and 12′	37.6	38.7	39.7	40.0	39.6
13 and 13′	47.5	43.9	42.3	42.6	42.2
14 and 14′	50.1	56.8	56.7	56.4	56.8
15 and 15′	21.8	24.5	24.3	24.2	24.3
16 and 16′	35.8	22.8	28.2	28.2	28.9
17 and 17′	220.8	63.6	56.1	56.3	55.9
18 and 18′	13.5	13.2	11.8	12.1	12.0
19 and 19′	19.3	19.2	19.3	18.9	19.3
20 and 20′	-	209.4	35.8	35.8	40.5
21 and 21′	-	31.5	18.7	12.2	21.2
22 and 22′	-	-	36.2	36.2	128.3
23 and 23′	-	-	23.8	23.8	129.3
24 and 24′	-	-	39.5	39.5	50.0
25 and 25′	-	-	28.0	28.0	31.8
26 and 26′	-	-	22.6	22.6	19.0
27 and 27′	-	-	22.8	22.8	21.1
28 and 28′	-	-	-	-	25.4
29 and 29′	-	-	-	-	12.2
2×CO (oxalate)	157.5	157.6	157.7	157.9	157.7

Several symmetrical steroidal oxalate dimers **2**, **4**, **6**, **8**, **10**, and **12** were synthesized from their respective alcohols using oxalyl chloride at room temperature using pyridine as a base [12]. One of the most pharmacologically important natural steroid hormones, estrone (**1**), which has a phenolic hydroxyl at C-3, was employed to synthesize a ring A-ring A dimer via an oxalic acid spacer. The reaction, carried out in pyridine and oxalyl chloride, resulted in the formation of the desired dimer bis(estra-1,3,5(10)-trien-17-on)-3-yl oxalate (**2**). The HRFABMS spectrum of **2** exhibited the $[M+H]^+$ ion at *m/z* 595.3059, which confirmed the formation of the dimer. The IR absorption bands at 1760 and 1740 cm^{-1} were for the oxalate carbonyls, and that at 1703 cm^{-1} was for the ketone carbonyl functionality at C-17. The ^1H NMR spectrum showed signals similar to those obtained from the starting material with the exception that the chemical shifts of the aromatic protons of ring A were more deshielded (δ 6.95, 6.98, and 7.31 as opposed to δ 6.46, 6.52, and 7.04 of **1**). The presence of an oxalate moiety in **2** was evident further in the ^{13}C NMR spectrum, which exhibited a signal at δ 156.1 for the two carbonyl carbons of the oxalate moiety. The ^{13}C chemical shift of C-3 and C-3′ quaternary carbons was shielded further (δ 147.9 as opposed to δ 154.9 of **1**) which confirmed the oxalate ester formation at C-3 and C-3′.

The IR spectra of oxalate dimers **4**, **6**, **8**, **10**, and **12** showed two characteristic absorption bands in the region of **1**, 764–1739 cm^{-1}. The ^1H and ^{13}C NMR spectra of these dimers demonstrated signals similar to those of the respective starting materials (**3**, **5**, **7**, **9**, and **11**) with the exception that significant downfield shifts of the signals for the protons and carbons adjacent to the oxalate group were observed. The ^{13}C NMR spectra (Table 1) displayed a signal within the region of δ 157.5–157.9 characteristics for the oxalate carbonyls.

In the ^1H NMR spectrum of compounds **4**, **6**, **8**, **10**, and **12** oxymethine protons of C-3 and C-3′ appeared as a multiplet at 4.73, 4.72, 4.71, 4.80, and 4.73 ppm correspondingly as opposite to the same shifts for the starting compounds **3**, **5**, **7**, **9**, and **11** with δ 3.52, 3.53, 3.52, 3.56, and 3.57 ppm respectively.

The ^{13}C chemical shifts of C-3 and C-3′ oxymethine carbons for compounds **4**, **6**, **8**, **10**, and **12** were deshielded multiplets observed in the range from 76.9 to 77.2 ppm (Table 1) as opposite to the shifts observed for the respective starting compounds **3**, **5**, **7**, **9**, and **11** with δ 71.4, 71.7, 71.8, 71.4, and 70.3 ppm.

EXPERIMENTAL

General Procedures. The steroid starting materials [estrone (**1**), DHEA (**3**), pregnenolone (**5**), cholesterol (**7**), 5α-cholestane (**9**), and stigmasterol (**11**)], dry pyridine, and oxalyl chloride were purchased from Aldrich and used as received. All chemicals and solvents were used throughout without further purification. The reactions were monitored and the purity of the products was assessed by thin-layer chromatography (TLC) performed on silica gel (Merck type 60) and visualized under UV illumination and/or by I_2 vapor. Melting points of the products were determined on a Gallen-kamp melting point apparatus. Infrared spectra (wave numbers in cm^{-1}) were recorded on an ATI Mattson Genesis FTIR spectrophotometer as KBr pellets. NMR spectra were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in ppm downfield from TMS, using the middle resonance of $CDCl_3$ (7.25 ppm for ^1H and 77.23 ppm for ^{13}C) as an internal standard and coupling constants J/Hz. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, m = multiplet), coupling constant(s), and integration and peak assignment. Mass spectroscopic analyses were performed at the EPSRC Mass Spectrometry Service at Swansea.

Synthetic Protocols and Experimental Data. *Bis*(estra-1,3,5(10)-trien-17-on)-3-yl oxalate (**2**). A stirred solution of estrone (**1**, 300 mg, 1.11 mmol) in pyridine (5 ml) was treated dropwise with oxalyl chloride (71 mg, 0.56 mmol) under N_2 at room temperature. 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 (**2**, 198 mg, 59%), mp: 254–256°C. IR ($CHCl_3$, ν_{max}, cm^{-1}): 3026 w (C-H), 2954 s (C-H), 2923 s (C-H), 2856 s (C-H), 1760 s (oxalate C=O), 1740 s (oxalate C=O), 1703 s (ketonic C=O), 1608 w (C=C), 1598 w (C=C), 1492 s, 1453 m, 1310 m, 1260 m, 1158 s (C-O), 1084 m, 1008 m, 911 m and 732 m.

^1H NMR (400 MHz, $CDCl_3$, J/Hz): δ 0.89 (s, 6H, 18-Me and 18-Me′, 6.95 (d, J = 1.8, 2H, H-4 and H-4′), 6.98 (dd, J = 1.8 and 8.2, 2H, H-2 and H-2′), 7.31 (d, J = 8.2, 2H, H-1 and H-1′).

^{13}C NMR (100 MHz, $CDCl_3$): δ 126.7 (C-1 and C-1′), 118.0 (C-2 and C-2′), 147.9 (C-3 and C-3′), 120.9 (C-4 and C-4′), 138.6 (C-5 and C-5′), 29.4 (C-6 and C-6′), 26.2 (C-7 and C-7′), 37.9 (C-8 and C-8′), 44.1 (C-9 and C-9′), 138.5 (C-10 and C-10′), 25.7 (C-11 and C-11′), 31.5 (C-12 and C-12′), 47.9 (C-13 and C-13′), 50.4 (C-14 and C-14′), 21.6 (C-15 and C-15′), 35.8 (C-16 and C-16′), 220.6 (C-17 and C-17′), 13.8 (C-18 and C-18′), 156.1 (2×O-CO). FABMS *m/z*: 595 $[M+H]^+$, 617 $[M+Na]^+$. HR FABMS: Found: 595.3058; calc 595.3059 for $C_{38}H_{43}O_6$. Similar synthetic procedure was followed for the synthesis of other dimers (**4**, **6**, **8**, **10** and **12**).

***bis*(Androst-5-en-17-on)-3β-yl oxalate (4)**. The title compound **4** [306 mg, 56%, mp: 281–282°C (decomp.)] was synthesized using DHEA (**3**, 500 mg, 1.73 mmol) in dry pyridine (6 ml) and oxalyl chloride (110 mg, 0.87 mmol). IR ($CHCl_3$, ν_{max}, cm^{-1}): 2954 s (C-H), 2889 s (C-H), 2858 s (C-H), 1764 s (oxalate C=O), 1740 vs (oxalate C=O), 1669 m (ketonic C=O), 1637 w (C=C), 1471 m, 1372 m, 1250 m, 1190 s (C-O), 1059 m, 966 m and 772 m.

^1H NMR (400 MHz, $CDCl_3$, J/Hz): δ 0.84 (s, 6H, 18-Me and 18′-Me), 1.02 (s, 6H, 19-Me and 19′-Me), 4.73 (m, 2H, 3-CH-O and 3′-CH-O), 5.39 (d, J = 5.1, 2H, 6-CH and 6′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 631 $[M+H]^+$, 653 $[M+Na]^+$. HR FABMS: Found: 631.3999; calc 631.3998 for $C_{40}H_{55}O_6$.

***bis*(Pregn-5-en-20-on)-3β-yl oxalate (6)**. The title compound **6** (269 mg, 45%, mp: 246–247°C) was obtained from pregnenolone (**5**, 550 mg, 1.74 mmol) in dry pyridine 6 ml) and oxalyl chloride (110 mg, 0.87 mmol). IR ($CHCl_3$, ν_{max}, cm^{-1}): 2954 s (C-H), 2889 s (C-H), 2858 s (C-H), 1762 s (oxalate C=O), 1740 vs (oxalate C=O), 1669 m (ketonic C=O), 1637 m (C=C), 1471 m, 1439 m, 1372 m, 1250 m, 1190 s (C-O), 1138 m, 1059 m, 1021 m, 966 m, 927 m and 772 w.

^1H NMR (400 MHz, $CDCl_3$): δ 0.58 (s, 6H, 18-Me and 18′-Me), 0.99 (s, 6H, 19-Me and 19′-Me), 2.08 (s, 6H, 21-Me and 21′-Me), 4.72 (m, 2H, 3-CH-O and 3′-CH-O), 5.36 (t, J = 2.1, 2H, 6-CH and 6′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 687 $[M+H]^+$, 709 $[M+Na]^+$. HRFABMS: Found: 687.4624; calc 687.4624 for $C_{44}H_{63}O_6$.

***bis*(Cholest-5-en)-3β-yl oxalate (8).** The title compound **8** (438 mg, 41%, mp: 220–221°C; lit. mp: 223–225°C [13]) was synthesized from cholesterol (**7**, 1.0 g, 2.59 mmol) in dry pyridine (10 ml) and oxalyl chloride (164 mg, 1.29 mmol).

IR ($CHCl_3$, ν_{max}, cm^{-1}): 2943 s (C-H), 2867 s (C-H), 1764 vs (oxalate ester C=O), 1742 vs (oxalate ester C=O), 1665 w (C=C), 1466 w, 1375 w, 1174 s (C-O), 912 w and 733 m.

^{1}H NMR (400 MHz, $CDCl_3$, J/Hz): δ 0.64 (s, 6H, 18-Me and 18′-Me), 0.82 (d, J = 6.8, 6H, 26-Me and 26′-Me), 0.83 (d, J = 6.8, 6H, 27-Me and 27′-Me), 0.88 (d, J = 6.5, 6H, 21-Me and 21′-Me), 0.99 (s, 6H, 19-Me and 19′-Me), 4.71 (m, 2H, 3β-CH-O and 3′β-CH-O), 5.36 (t, J = 2.7, 2H, 6-CH and 6′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 827 $[M+H]^+$, 849 $[M+Na]^+$. HRFABMS: Found: 827.6919; calc 827.6917 for $C_{56}H_{91}O_4$.

***bis*(5α-Cholestan)-3β-yl oxalate (10)**. The title compound **10** (451 mg, 42%, mp: 209–210°C; lit. mp: 212°C [14]) was obtained from 5α-cholestan-3β-ol (**9**, 1.0 g, 2.57 mmol) in dry pyridine (10 ml) and oxalyl chloride (164 mg, 1.29 mmol). IR ($CHCl_3$, ν_{max}, cm^{-1}): 2930 s (C-H), 2863 s (C-H), 1764 s (oxalate C=O), 1745 vs (oxalate C=O), 1471 w, 1373 w, 1183 s (C-O), 997 m, 927 m, 755 m and 607 w.

^{1}H NMR (400 MHz, $CDCl_3$, J/Hz): δ 0.60 (s, 6H, 18-Me and 18′-Me), 0.81 (d, J = 6.5, 6H, 26-Me and 26′-Me), 0.82 (d, J = 6.8, 6H, 21-Me and 21′-Me), 0.85 (d, J = 6.5, 6H, 27-Me and 27′-Me), 0.79 (s, 6H, 19-Me and 19′-Me), 4.80 (m, 2H, 3-CH-O and 3′-CH-O) and ^{13}C NMR (Table 1). FABMS: *m/z* 831 $[M+H]^+$, 853 $[M+Na]^+$. HRFABMS: Found: 831.7228; calc 831.7229 for $C_{56}H_{95}O_4$.

***bis*(Stigmasta-5,22t-dien)-3β-yl oxalate (12)**. The title compound **12** (491 mg, 46%, mp: 184–185°C) was synthesized from stigmasterol (**11**, 1.0 g, 2.42 mmol) in dry pyridine (10 ml) and oxalyl chloride (154 mg, 1.21 mmol). IR ($CHCl_3$, ν_{max}, cm^{-1}): 2947 s (C-H), 2872 (C-H), 1762 s (oxalate C=O), 1739 vs (oxalate C=O), 1665 w (C=C), 1457 w, 1368 w, 1195 s (C-O), 970 m and 758 w.

^{1}H NMR (400 MHz, $CDCl_3$, J/Hz): δ 0.66 (s, 6H, 18-Me and 18′-Me), 0.75 (d, J = 7.2, 6H, 26-Me and 26′-Me), 0.77 (t, J = 7.5, 6H, 29-Me and 29′-Me), 0.81 (d, J = 6.5, 6H, 27-Me and 27′-Me), 0.97 (s, J = 6.1, 6H, 21-Me and 21′-Me), 1.00 (s, 6H, 19-Me and 19′-Me), 1.49 (m, 2H, 25-CH and 25′-CH), 4.73 (m, 2H, 3β-CH-O and 3β-CH-O), 4.98 (dd, 2H, J = 8.5 and 15.1, 23-CH and 23′-CH), 5.12 (dd, 2H, J = 8.5 and 15.1, 22-CH and 22′-CH), 5.36 (d, J = 5.1, 2H, 6-CH and 6′-CH) and ^{13}C NMR (Table 1). FABMS: *m/z* 879 $[M+H]^+$, 901 $[M+Na]^+$. HRFABMS: Found: 879.7229; calc 879.7229 for $C_{60}H_{95}O_4$.

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