Steroids 76 (2011) 232–237

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of new symmetrical bis-steroidal pyrazine analogues from diosgenin

Khaled Q. Shawakfeh*, Naim H. Al-Said

Department of Applied Chemical Sciences, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan

ARTICLE INFO

Article history:
Received 23 August 2010
Received in revised form 4 October 2010
Accepted 6 October 2010
Available online 15 October 2010

Dedicated to Prof. John R. Williams for his continuos support.

Keywords:
Steroidal
Pyrazine
Diosgenin
Dimers
Oxysterol

ABSTRACT

New symmetrical bis-steroidal pyrazine dimers that are cephalostatins/ritterazines analogues have been prepared easily from a cheap, readily available natural steroid (diosgenin). These dimers were obtained by classical, condensation of α-amino ketones in order to construct the pyrazine rings. The three dimers differ in the functionalized diosgenin: (25R)-5α,6β-dihydroxy-5α-spirosta-3-one, (25R)-4,5α-epoxy-5β-spirosta-3,6-dione and (25R)-5α-hydroxy-5α-spirosta-3,6-dione respectively.

1. Introduction

Marine species such as sponges, coelenterates, mollusks and echinoderms are a rich source of a large variety of polyhydroxy steroids [1]. The synthesis of polyhydroxy sterols has been a challenging subject for several years. Diosgenin is the steroidal saponin that is isolated from the Mexican yam. Due to its structural similarity to estrogen and progesterone precursors, diosgenin exhibts estrogenic, progesterogenic and anti-inflammatory effects [2]. In addition, diosgenin was used for the synthesis of nearly 50% of the total steroid drugs in the world [2]. In 1988, a family of dimeric steroid-pyrazine alkaloids and dimers was discovered by the Pettit group [3]. Latter, as a continuation of their work on these dimers, during the period from 1988 to 1998, the same group reported the isolation and identification of compounds of this class, cephalostatines 2-19 [4–6]. For example Cephalostatin 1 (Fig. 1) was proved to be one of the most powerful cancer cell growth inhibitors with an ED_{50} value of 0.1–0.001 pM. This exceptional activity of cephalostatins has led to interest in the synthesis of compounds and their analogues as potential anti-tumor agents [7].

The cephalostatin **1** and the closely related ritterazines B share many common structural features in which two highly oxygenated C_{27} steroidal units are fused via a pyrazine ring at C-2 and C-3 and both chains of the steroidal units form spiroketals [8]. The cephalostatins in general are more oxygenated on the right side, whereas the ritterazines have the more oxygenated left side. These natural steroid dimers possess extremely potent inhibitory activity against a series of human cancer cell lines and the murine P388 lymphocytic leukemia cell line [7b,9]. Since the natural sources of these compounds are extremely limited, and also the yield of these compounds in nature is rather poor, the syntheses of a number of various analogues of cephalostatines and ritterazines have been reported in the literature [10–12].

There are many synthetic approaches for the central pyrazine ring. Some of them describe the synthesis of unsymmetrical dimers [13]. The symmetrical dimeric steroid-pyrazines can be obtained by the classical condensation of α-amino ketones. This route is actually, the most efficient method of pyrazine rings construction. The intermediate steroidal 2α-amino-3-ketones are available by reduction of the corresponding 3-ketones with a nitrogen containing substituent at C-2, such as azido [14] nitro [15], hydroxyimino [16] and enamino [17] groups. The initially formed 2α-amino-3-ketones undergo spontaneous dimerization to a mixture of dihydropyrazines, which are then oxidized by air to give pyrazine dimers [3].

A new methodology for the synthesis for four new cephalostatin analogues was reported by Shawakfeh et al. [18]. The main component of the steroid mixture was the known diosgenin, which has a spiroketal ring in the side chain. As a continuation of our studies and due to the structural similarities between cephalostatin/rittarazine and diosgenin, we report here the synthesis of three new polyoxgenated symmetrical bis-steroidal pyrazine analogues that are expected to show more biological effect due to the increased presence of polar functional groups.

* Corresponding author. Tel.: +962 2 7201000; fax: +962 2 7095014.
E-mail address: shawakfa@just.edu.jo (K.Q. Shawakfeh).

0039-128X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2010.10.002

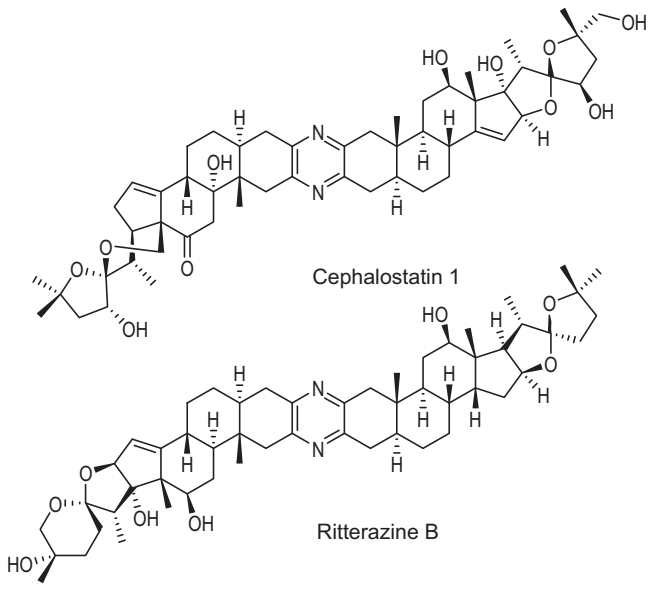


Fig. 1. Structure of a cephalostatin **1** and ritterazine B.

2. Experimental

2.1. General

Melting points (mp) were determined on an electrothermal digital melting point apparatus and are uncorrected. All the starting materials and reagents were obtained from commercial sources and were used without further purification. FT-IR spectra were recorded on a Nicolet-Impact 410 spectrophotometer. Both ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-400 instruments. The chemical shifts (δ) are reported in ppm relative to TMS used as an internal standard.

2.2. Chemical synthesis

2.2.1. (25R)-5-En-spirosta-3-one (**2**)

Pyridinum chlorochromate (1.7 g, 4.0 mmol) was added to a mixture of powdered $CaCO_3$ (4.0 g, 4.0 mmol) and **1** (2.0 g, 4.8 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was stirred for 30 min. The reaction mixture was diluted with diethyl ether (50 mL) and filtered through a short column of florisil. The solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (10% ethyl acetate in hexane) to give a ketone **2** as a white solid. Yield: 1.6 g (80%), mp 194–195 °C, lit. mp 194–196 °C [14]. IR (KBr): 2961, 1726 and 1681 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.3 (s, 1H, C6), 4.4 (dt, J = 6.8, 8.0 Hz, 1H, C16α), 3.48 (m, 1H, C26α), 3.38 (*t*, J = 10.7 Hz, 1H, C26β). ^{13}C NMR ($CDCl_3$): δ 211 (C3), 140.6 (C5), 122 (C6), 80.1 (C17), 66.7 (C26), 109 (C22), 62.5 (C17).

2.2.2. (25R)-5,6-α-Epoxy-5α-spirosta-3-one (**3**)

A solution of **2** (1.0 g, 2.4 mmol) in dichloromethane (25 mL) was cooled to 0 °C and a solution of m-chloroperoxybenzoic acid (1.8 g, 10.4 mmol) in dichloromethane (20 mL) was added dropwise. The reaction mixture was stirred at room temperature for 22 hours, and successively washed with 5% solution of Na_2SO_3, saturated $NaHCO_3$, and water. Extraction with $CHCl_3$ (2 × 50 mL) and then the organic layer was dried over Na_2SO_4, evaporated to dryness under reduced pressure. The crude product was purified by crystallization from acetone and gave a white solid of **3**. Yield: 0.5 g (95%), mp 171–173 °C. IR [KBr]: 2943, 1715, 1681, 1059 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.39 (dt, J = 6.8, 8.1 Hz, 1H, C16α), 3.41 (m, 1H, C26α), 3.3 (*t*, J = 10.8 Hz, 1H, C26β), 2.94 (*d*, J = 4.5 Hz, 1H, C6β), 1.18 (s, 3H, C19). ^{13}C NMR ($CDCl_3$): δ 211 (C3), 109 (C22), 81 (C16), 67 (C26), 65 (C5), 59 (C6). Anal. Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 74.98; H, 9.38.

2.2.3. (25R)-5α, 6β-dihydroxy-5α-spirosta-3-one (**4**)

To a solution of **3** (1.0 g, 2.33 mmol) in acetone (60 mL) and water (20 mL), was added perchloric acid (70%, 0.4 mL) with vigorous stirring for 2 days. The reaction mixture was extracted with chloroform (3 × 25 mL) and the combined organic layer was dried over Na_2SO_4, concentrated to dryness and purified by column chromatography (30% ethyl acetate/hexane) to give a white solid of **4**. Yield: 0.55 g (50%), mp 142–144 °C. IR [KBr]: 3327, 2957, 1712, 1681, 1058 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.37 (d*t*, J = 6.6, 8.1 Hz, 1H, C16α), 3.5 (*t*, J = 2.7 Hz, 1H, C6). ^{13}C NMR ($CDCl_3$): δ 212 (C3), 109 (C22), 81 (C16), 67 (C26), 79 (C6), 75 (C5). Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48.Found: C, 72.55; H, 9.43.

2.2.4. (25R)-2α-Bromo-5α,6β-dihydroxy-5α-spirosta-3-one (**5**)

To a solution of dihydroxy ketone **4** (0.2 g, 0.45 mmol) in THF (10 mL) at room temperature, phenyl trimethylammonium perbromide (PTAB, 0.22 g, 0.59 mmol, 1.3 equiv) in THF (7 mL) was added rapidly. The resulting orange solution deposited a copious precipitate which faded to a beige color within 10 min. The solution was quenched with brine solution (10 mL), extracted with $CHCl_3$ (2 × 20 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (30% ethyl acetate/hexane) to give a pale yellow solid of bromodihydroxy ketone **5**. Yield: 0.16 g (69%), mp 147–150 °C. IR (KBr, cm^{-1}): 3319, 2951, 1687 and 1053. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.76 (dd, J = 7.2, 5.2 Hz, 1H, C2β, 4.37 (dt, J = 6.8, 7.7 Hz, 1H, C16α), 3.57 (*t*, J = 2.7 Hz, 1H, C6α). ^{13}C NMR ($CDCl_3$): δ 211 (C3), 109 (C22), 81 (C16), 79 (C6), 76 (C5), 67 (C26). Anal. Calcd. for $C_{27}H_{41}O_5Br$: C, 61.71; H, 7.86. Found: C, 61.57; H, 7.68.

2.2.5. Di (25R-6α-4,6β-dihydroxy-5α-spirostano[2,3-b:2′,3′-e]) pyrazine (**6**)

The bromodihydroxy ketone **5** (0.2 g, 0.38 mmol) was dissolved in DMF (30 mL) and a few mg of KI were added followed by addition of NaN_3 (0.24 g, 3.8 mmol, 10 equiv.). The mixture was stirred for 3 h at 50 °C. The solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL) and the resulting solution was washed with brine solution (2 × 20 mL), dried over Na_2SO_4 and evaporated to give the azido ketone intermediate. To the intimate mixture of azido ketone in THF (5 ml) was added triphenylphosphine (0.33 g, 1.24 mmol, 3 equiv.) under N_2. The solution was stirred for 5 h under N_2 until the evolving gas from the solution ceased. Then THF (5 mL) and water (0.2 mL, 11 mmol) were added and the reaction mixture was stirred overnight. After concentration, the yellow residue was azeotroped with toluene and absolute ethanol (10 mL) and TsOH (catalytic amount) were added. The orange mixture was stirred vigorously at room temperature under atmospheric pressure for further 2 days. The fine solid was filtered over a bed of celite and washed with $CHCl_3$ followed by evaporation to give a dimeric pyrazine **6**. The product was purified by crystallization from methanol and give pure dimeric pyrazine **6**. Yield: 0.11 g, 64%), m.p. 260 °C(dec). IR (KBr, cm^{-1}): 3383, 2950, and 1417. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.40 (dt, J = 6.8, 8.1 Hz, 1H, C16α), 3.46 (*t*, J = 2.7 Hz, 1H, C6α), 1.09 (s, 3H, C19). ^{13}C NMR ($CDCl_3$): δ 143, 141 (pyrazine carbons), 109 (C22), 81 (C16), 79 (C6), 76 (C5), 67 (C26). Anal. Calcd. for $C_{54}H_{80}N_2O_8$: C, 73.27; H, 9.11; N, 3.16. Found: C, 73.07; H, 8.98; N, 3.01.

2.2.6. (25R)-4-en-Spirosta-3,6-dione (**7**)

Freshly prepared Jones reagent was added dropwise to a solution of **1** (2.0 g, 4.82 mmol) in acetone (150 mL) at 10 °C. The reaction

mixture was stirred below 20 °C for 30 min. The reaction mixture was washed with brine solution, extracted with $CHCl_3$ (3 × 25 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (20% ethyl acetate/hexane) to give a white solid of pure **7**. Yield: 1.95 g (95%), mp. 190–193 °C, lit. mp 190–192 °C [19]. IR (KBr, cm^{-1}): 2943, 1681, 1610, and 1450. ^1H NMR (400 MHz, $CDCl_3$): δ 6.3 (s, 1H, C4), 4.36 (dt, J = 6.8, 8.1 Hz, 1H, C16-αH), 3.45 (m, 2H, C26), 3.37 (t, 2H, C26). ^{13}C NMR (400 MHz, $CDCl_3$): δ 202 (C3), 199 (C6), 163 (C5), 127 (C4), 109 (C22), 80.6 (C16), 67 (C26), 62.7 (C17), 56 (C14).

*2.2.7. (25R)-4,5α-Epoxy-5β-spirosta-3,6-dione (**8**)*

To a solution of diketone **7** (0.2 g, 0.47 mmol) in MeOH (20 mL) at 15 °C, hydrogen peroxide (30% H_2O_2, 5.0 mL) was added rapidly followed by dropwise addition of sodium hydroxide solution (NaOH, 4 M, 3.0 mL). The reaction mixture was stirred keeping the temperature below 15 °C for 1 h, then concentrated followed by extraction with $CHCl_3$ (2 × 20 mL). The extract was washed with water (2 × 15 mL), dried over Na_2SO_4, concentrated to get a white solid. The residue was purified by column chromatography (20% ethyl acetate/hexane) to give a white solid of pure **8** (0.13 g, 63%). M.p. 167–169 °C. IR (KBr): 2949, 1726, 1705, and 1054 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.37 (dt, J = 6.6, 8.1 Hz, 1H, C16α), 2.98 (s, 1H, C4β), 3.48 (m, 2H, C26), 3.35 (t, 2H, C26), 1.26 (s, 3H, C19). ^{13}C NMR ($CDCl_3$): δ 203 (C3), 201 (C6), 109 (C22), 81 (C16), 70 (C5), 67 (C26), 58 (C4). Anal. Calcd. for $C_{27}H_{38}O_5$: C, 73.27; H, 8.65. Found: C, 72.97; H, 8.58.

*2.2.8. (25R)-2α-Bromo-4,5α-epoxy-5β-spirosta-3,6-dione (**9**)*

Procedure as described for **5** gave a pale yellow solid of bromodione **9**. Yield: 0.17 g (72%), mp 170–172 °C. IR (KBr, cm^{-1}): 2951, 1720, 1704, 1054, and 838 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.68 (dd, J = 6.9, 5.2 Hz, 1H, C2β), 4.36 (dt, J = 6.6, 8.1 Hz, 1H, C16α), 3.88 (s, 1H, C4β), 3.48 (m, 2H, C26), 3.35 (t, 2H, C26), 1.26 (s, 3H, C19). ^{13}C NMR ($CDCl_3$): δ 209 (C3), 206 (C6), 109 (C22), 81 (C16), 70 (C5), 69 (C4), 54 (C2). Anal. Calcd. for $C_{27}H_{37}O_5Br$: C, 62.19; H, 7.15. Found: C, 61.97; H, 7.07.

*2.2.9. Di (25R-6 oxo-4,5α-epoxy-5β-spirostano[2,3-b:2′,3′-e]) pyrazine (**10**)*

Procedure as described for **6** gave a white solid of pure dimeric pyrazine **10**. Yield: 0.12 g (65%), m.p. 300 °C(d). IR (KBr, cm^{-1}): 2922, 1719, and 1417. ^1H NMR (300 MHz, $CDCl_3$): δ 4.40 (dt, J = 6.8, 8.1 Hz, 1H, C16α), 3.84 (s, 1H, C4β), 1.07 (s, 3H, C19). ^{13}C NMR ($CDCl_3$): δ 203 (C6), 142, 141 (pyrazine carbons), 109 (C22), 81 (C16), 70 (C5), 67 (C4). Anal. Calcd. for $C_{54}H_{72}N_2O_8$: C, 73.94; H, 8.27; N, 3.19. Found: C, 73.69; H, 8.18; N, 3.04.

*2.2.10. (25R)-5,6α-Epoxy-5α-spirosta-3β-ol (**11**)*

Procedure as described for **3** gave a white solid of the epoxy **11**. Yield: 0.95 g (92%), mp 200–202 °C, lit. m.p 202–204 [20]. IR (KBr, cm^{-1}): 3510, 3400, 2950, 1645, and 1053. ^1H NMR (300 MHz, $CDCl_3$): δ 4.36 (m, 1H, C16), 3.90 (m, 1H, C3,), 3.48 (m, 2H, C26), 2.9 (d, J = 14 Hz, 1H, C6), 1.08 (s, 3H, C19), 0.76 (s, 3H, C18). ^{13}C NMR (300 MHz, $CDCl_3$: δ 109 (C22), 81 (C16), 70 (C3), 67 (C26), 66 (C5), 59 (C6).

*2.2.11. (25R)-5α-Hydroxy-5α-spirosta-3,6-dione (**12**)*

Procedure as described for **7** but with a solution of **11** (2.0 g, 4.65 mmol) gave a white solid of pure **12**. Yield: 1.8 g (87%), mp 191–192 °C. IR (KBr, cm^{-1}): 3346, 2949, 1713, and 1705.^1H NMR (300 MHz, $CDCl_3$): δ 4.48 (m, 1H, C16), 3.48 (t, 2H, C26), 2.80–2.90 (s, 2H, C4), 2.26 (m, 2H, C2). ^{13}C NMR (300 MHz, $CDCl_3$): δ 210 (C6), 209 (C3), 109 (C22), 82 (C5), 67 (C26), 63 (C17), 56 (C14). Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 72.77; H, 8.95.

*2.2.12. (25R)-2α-Bromo-5α-hydroxy-spirosta-3,6-dione (**13**)*

Procedure as described for **5** gave a white solid of bromo diketone **13**. Yield: 0.26 g (74%), mp 195–197 °C. IR (KBr, cm^{-1}): 3340, 2951, 1711, 1705, and 840. ^1H NMR (300 MHz, $CDCl_3$): δ 4.70 (1H, dd, H2β), 4.45 (1H, dt, 16α), 3.48 (2H, m, H 26). ^{13}C NMR (300 MHz, $CDCl_3$): δ 209 (C3), 208 (C6), 109 (C22), 82 (C5), 54 (C2). Anal. Calcd. for $C_{27}H_{39}O_5Br$: C, 61.95; H, 7.51. Found: C, 61.77; H, 7.45.

*2.2.13. Di (25R-3,6 dioxo-5α-hydroxy-5α-spirostano[2,3-b:2′,3′-e]) pyrazine (**14**)*

Procedure as described for **6** gave pure dimeric pyrazine **14**. Yield: 0.16 g (62%), m.p. 290 °C(d). IR (KBr, cm^{-1}): 3353, 2944, 1706, and 1404. ^1H NMR (300 MHz, $CDCl_3$): δ 4.4 (dt, 1H, C16α), 3.45 (m, 2H, C26), 1.14 (s, 3H, C19). ^{13}C NMR (300 MHz, $CDCl_3$): δ 203 (C6), 139, 138 (pyrazine carbons), 108 (C22), 79 (C5), 67 (C26). Anal. Calcd. for $C_{54}H_{76}N_2O_8$: C, 73.60; H, 8.69; N, 3.18. Found: C, 73.52; H, 8.60; N, 3.06.

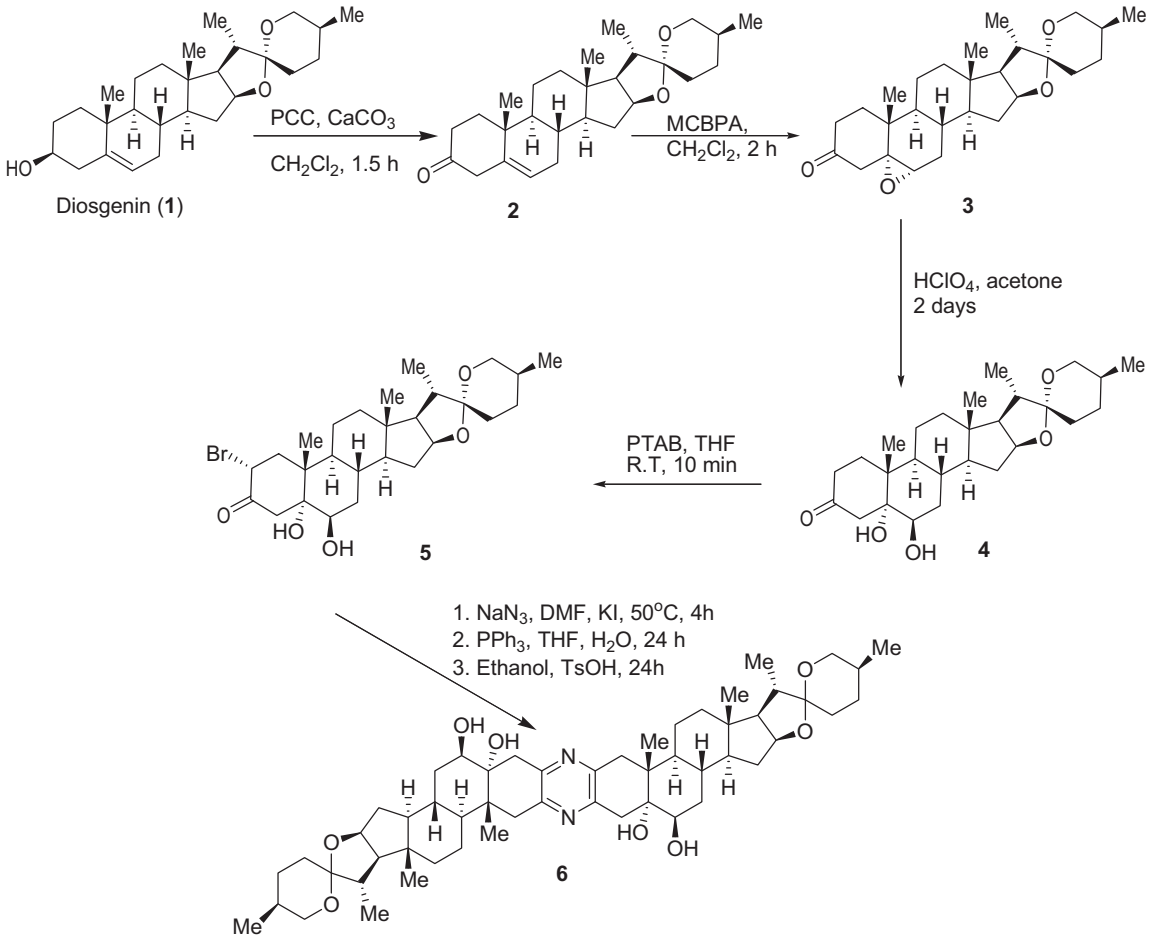
3. Results and discussion

In this paper, the synthesis of dimer **6**, which has two hydroxyl groups at C5 and C6, was carried out, as shown in Scheme 1. Oxidation of diosgenin **1** with PCC in the presence of $CaCO_3$ gave the known ketone **2** [18]. Next, the reaction of m-chloroperoxybenzoic acid (mCPBA) with the ketone **2** gives the 5α, 6α-epoxide **3** with the epoxidation taking place predominantly from the α-face of the molecule. The selective epoxidation from the α-face was attributed to the equatorial attack of mCPBA to the double bond of diosgenin is destabilized by the steric interaction between the reagent and the axial CH_3 group at C-19. The ^1H NMR spectrum for product **3** shows doublet of triplet peak at 4.39 ppm (J = 6.6, 8.1) for one α proton at C16 and a doublet peak at 2.94 ppm (J = 4.5 Hz) for one β proton at C6. The ^{13}C NMR spectrum shows new peaks at 59 and 65 ppm for C6 and C5, respectively. Moreover, the signal representing C3 was observed at 211 ppm. The protonation of the epoxide **3** will lead to the formation of oxonium intermediate, which is susceptible to nucleophilic attack by water will lead to the formation of the diol **4**. The ^1H NMR spectrum for product **4** shows doublet of triplet peak at 4.37 ppm (J = 6.6, 8.1) for one α proton at C16 and a triplet peak at 3.5 ppm (J = 2.7 Hz) for one α proton at C6. The ^{13}C NMR spectrum shows new peaks at 79 and 76 ppm for C6 and C5, respectively, in addition to the peak at 212 ppm for C3.

Selective bromination at C2 by phenyl trimethylammonium perbromide (PTAB) gave the α-bromo ketone **5** as the thermodynamic product. The ^1H NMR spectrum shows doublet of doublets at 4.76 ppm (J = 7.2, 5.2 Hz) for β proton at C2 and doublet of triplet peak at 4.37 ppm for one α proton at C16. The ^{13}C NMR spectrum shows a new peak at 54 ppm for C2, in addition to the peaks at 211, 79 and 76 ppm for C3, C6 and C5 respectively.

Next, completion of the total synthesis required direct conversion of α-bromodione **5** into the targeted bis-steroidal pyrazine dimer **6**. Therefore substitution of bromide with an azido group using sodium azide in the presence of catalytic amount of sodium iodide furnished the corresponding α-azido ketone. This α-azido ketone was not stable at room temperature and it was reduced by aqueous triphenyl phosphine (Staudinger reaction) in THF for 24 h. Addition of a catalytic amount of p-toluenesulfonic acid in ethanol and oxidation in air afforded the bis steroidal pyrazine dimer **6** in 64% yield. The ^1H NMR spectrum shows a triplet peak at 3.46 ppm (J = 2.7 Hz) for one α proton at C6 and a doublet of triplet peak at 4.4 ppm for one α proton at C16. The ^{13}C NMR spectrum shows two peaks at 141 and 143 ppm characteristics for the pyrazine carbons, in addition to the C5 and C6 peaks at 76 and 79 ppm respectively.

Oxysterols represent a class of potent regulatory molecules with remarkably diverse, important biological activities [19]. They

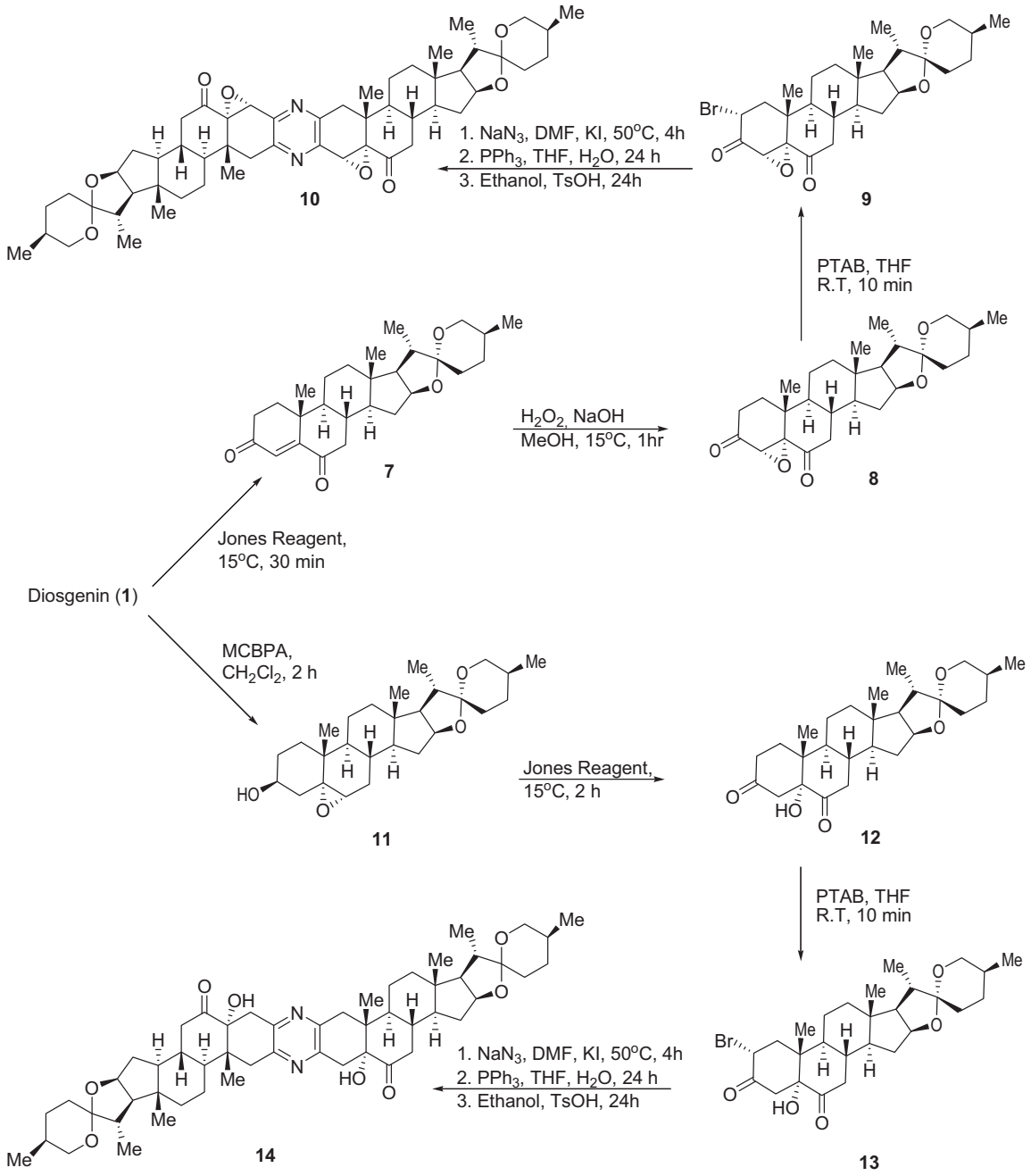


Scheme 1. Synthesis of dimer 6.

exhibit a number of biological activities, including inhibition of cellular proliferation and cytotoxicity associated with induction of apoptosis [20]. For this reason, we decided to synthesize new polyoxgenated pyrazine dimers taking advantages of epoxidation methodology presented in Scheme 1.

Here, we present a chemically simple, rapid, economical and diverse one-pot synthesis for generation of Δ^4-3, 6-dione functionalized diosgenin as shown in Scheme 2. Utilization of the modified Jones oxidation methodology afforded 4-ene-3, 6-dione compound **7** in 95% yields. The ^{1}H NMR spectrum for compound **7** showed a singlet resonance signal at 6.3 ppm for the C4 proton. The ^{13}C NMR spectrum of **7** showed two new peaks at 202 and 199 ppm for carbonyl groups at C3 and C6, respectively. The nature of the double bond values changed significantly for the C4 and C5 value to 127 and 163 ppm respectively. Both the ^{1}H and ^{13}C NMR melting points were consistent with the literature reported values [21,22]. Having successfully prepared compound **7**, we next turned to introduce an epoxide ring at C4/C5, thus compound **7** was epoxdized with 30% H_2O_2 and NaOH solution in methanol to give the 4α, 5α-epoxide **8**, with the epoxidation taking place predominantly from the α-face of the molecule. The ^{1}H NMR spectrum for product **8** showed doublet of triplet peak at 4.37 ppm (J = 6.6, 8.1) for one α proton at C16 and a singlet peak at 2.98 ppm for one β proton at C4, in addition to the significantly shifted singlet peak at 1.26 ppm for three protons at C19. The ^{13}C NMR spectrum shows two peaks at 203 and 201 ppm for carbonyl groups at C3 and C6, respectively. The epoxide carbons at C4 and C5 appear at 67 and 70 ppm, respectively. These data are consistent with reported values for systems with 4α,5-epoxy-5α and 4β,5-epoxy-5β-sterol [23]. Following the same protocol for compound **4** by selective bromination at C2 of compound **8** by phenyl trimethylammonium perbromide (PTAB) gave the α-bromodione epoxide **9** as the thermodynamic product. The ^{1}H NMR spectrum shows doublet of doublets at 4.68 ppm (J = 6.9, 5.2 Hz) for β proton at C2 and doublet of triplet peak at 4.36 ppm for one α proton at C16. The ^{13}C NMR spectrum shows a new peak at 54 ppm for C2, in addition to the peaks at 209, 206, 69 and 70 ppm for C3, C6, C4 and C5 respectively. The dimerization of the bromoketone **9** involved two steps to achieve the formation of the pyrazine dimer **10**. The first step is the substitution of the bromo atom with an azido group using sodium azide in the presence of catalytic amount of sodium iodide. The next step is reduction to α-amino ketone by triphenylphosphine in dry THF followed by addition of water to hydrolyze the aza-Wittig intermediate. The resulting amino ketone intermediate was stirred in ethanol and toluene containing a catalytic amount of p-toluenesulfonic acid, and open to the atmosphere to facilitate aromatization to yield the pyrazine dimer **10** in good overall yield. The ^{1}H NMR spectrum shows a doublet of triplet peak at 4.4 ppm for one α proton at C16 and a singlet peak at 3.84 ppm for one proton at C4. The ^{13}C NMR spectrum shows two peaks at 141 and 142 ppm characteristics for the pyrazine carbons. It also shows the epoxide carbon peaks at 67 and 70 ppm and the carbonyl carbon peak at 203 ppm.

For the last dimer, we decided to have free hydroxyl group at C5 keeping the ketone functionality at C6 as outlined in Scheme 2. Diosgenin **1** was treated with MCPBA and gave epoxysterol **11** in 92% yields. The ^{1}H and ^{13}C NMR as well as melting point were consistent with literature reported values [2]. The next step was to increase the oxygen content by further oxidation in order to



Scheme 2. Synthesis of dimers 10 and 14.

generate new oxysterols. Therefore, Jones oxidation of epoxysterol **11** furnished in excellent yield the diketosterol **12**. The ^{1}H NMR spectrum shows two peaks at 2.9 and 2.26 ppm for the C4 and C2 methylene protons. The ^{13}C NMR spectrum shows three new peaks at 210, 219 and 82 ppm for ca rbonyl carbons at C3 and C6 and for the 3°-alcohol carbon at C5, respectively. Selective bromination at C2 by phenyl trimethylammonium perbromide (PTAB) gave the α-bromodione **13** as the thermodynamic product. Interestingly bromination did not occur at C7 α to the C6 ketone. The ^{1}H NMR spectrum shows doublet of doublets at 4.70 ppm for β proton at C2 and doublet of triplet peak at 4.45 ppm for one α proton at C16. The ^{13}C NMR spectrum shows a new peak at 54 ppm for C2, in addition to the peaks at 209, 208 and 82 ppm for C3, C6 and C5 respectively.

As mentioned earlier, the dimerization steps required direct conversion of α-bromodione **13** into our target which is the bis-steroidal pyrazine dimer **14**. In our case, substitution by sodium azide in the presence of catalytic amount of sodium iodide, followed by addition of a catalytic amount of p-toluenesulfonic acid in ethanol and further oxidation in air afforded bis steroidal pyrazine dimer **14** in 62% yield. The ^{1}H NMR spectrum shows a doublet of triplet peak at 4.4 ppm for one α proton at C16 and a singlet peak at 1.24 ppm for three protons at C19. The ^{13}C NMR spectrum shows two peaks at 139 and 138 ppm characteristics for the pyrazine carbons. It also shows the 3°-alcoholic carbon peak at 79 ppm and the carbonyl carbon peak at 203 ppm.

4. Conclusion

A convenient synthesis for three new symmetrical bis-steroidal pyrazine dimers from a cheap, available and natural steroid (dios-

genin) is reported. These polyoxygenated symmetrical bis steroidal pyrazine analogues were obtained by classical condensation of α-amino ketones that is the most efficient method of pyrazine rings construction.

Acknowledgements

We would like to thank Jordan University of Science and Technology for the opportunity to do this research during a sabbatical leave at University of Dammam-Saudia Arabia. Also many thanks to Dr. Abedlatif Ibdah at King Fahed for Petroleum and Minerals for doing some spectroscopic analysis.

References

[1] Silpa K, Mirza B, Umesh D, Girism T. Differential behavior of (25*R*)-5,6-epoxyspirostan-22α-*O*-3β-ol and (25*R*)-5,6-epoxyspirostan-22α-*O*-3β,4β-diol toward Dowex. Steroids 1996;61:290–5.
[2] Eunsook M, Jungwan K. Epoxidation of diosgenin, 25(R)-1,4,6-spirostatrien-3-one and 25(R)-4,6-spirostadien-3β-ol. Molecules 2003;8:886–93.
[3] Pettit GR, Inoue M, Herald DL, Krupa TS. Isolation and synthesis of the powerful cell growth Inhibitor Cephalostatin. J Am Chem Soc 1988;110:2006.
[4] Pettit JR, Xu JP, Schmidt JM. Use of ^{15}N-HMBC NMR techniques to determine the orientation of the steroidal units in ritterazine A. Bioorg Med Chem Lett 1995;5:2027–32.
[5] Pettit JR. Marine animal and terrestrial plant anticancer constituents. Pure Appl Chern 1994;66:2271–81.
[6] Pettit GR, Tan R, Xu J-P, Ichihara Y, Williams MD, Boyd MR. Antineoplastic agents. 398. Isolation and structure elucidation of cephalostatins 18 and 19. J Nat Prod 1998;61:955–8.
[7] (a) Jeong JU, Seongkon K, Fuchs PL. Biomimetic total syntheses of (+)-cephalostatin 7, (+)-cephalostatin 12, and (+)-ritterazine K[1]. J Am Chem Soc 1995;117:10157–8;
(b) Jautelat R, Mullur-Fahrnow A, Winterfeldt E. A novel oxidative cleavage of the steroidal skelton. Chem Eur J 1999;5:1226–33;
(c) Thomas GL, Chuanxing G, Suhhakar B, Michael RB, Fuchs PL. Interphylal product splicing: the first total syntheses of cephalostatin 1, the north hemisphere of ritterazine G, and the highly active hybrid analogue, ritterostatin G(N)1(N). J Am Chem Soc 1998;120:692–707.
[8] Moser BR. Review of cytotoxic cephalostatins and ritterazines: isolation and synthesis. J Nat Prod 2008;71:487–91.
[9] Li Y, Dias JR. Dimeric and oligomeric steroids. Chem Rev 1997;97:283–304.
[10] Nahar L, Saker SD, Turner AB. A review on synthetic and natural steroid dimers: 1997–2006. Curr Med Chem 2007;14:1349–70.
[11] Lee S, LaCour TG, Fuchs PL. Chemistry of trisdecacyclic pyrazine antineoplastics: the cephalostatins and ritterazines. Chem Rev 2009;109:2275–314.
[12] Fukuzawa S, Matsunaga S, Fusetani N. Isolation and structure elucidation of ritterazines B and C, highly cytotoxic dimeric steroidal alkaloids, from the tunicate Ritterella tokioka. J Org Chem 1995;60(3):608–14.
[13] Drogemuller M, Flessner T, Jautelat R, Scholzf U, Winterfeldt E. Synthesis of cephalostatin analogues by symmetrical and non-symmetrical routes. Eur J Org Chem 1998:2811–31.
[14] Pan Y, Merriman RL, Tanzar LR, Fuchs PL. Synthesis and pharmacological evaluation of nonacyclic and trisdecacyclic pyrazines related to cephalostatin. Bioorg Med Chem Lett 1992;2:967–72.
[15] Morzycki AW, Gryszkiewicz A, Lotowski Z, Szczepek WJ. Reduction of 2-nitro-5α-cholestan-3-one, its Enol tautomer and 2-nitro-5α-cholest-2-en-3-amine derivatives. Synthesis of bis-steroidal pyrazines. Collec Czech Chem Commun 1998;63:1589–96.
[16] Smith HE, Hicks AA. Optically active amines. XII. Synthesis and spectral properties some optically active alpha-oximino ketones and alpha-amino ketone hydrochlorides. Dimerization of alpha-amino ketones. J Org Chem 1971;36:3659–68.
[17] Kramer A, Ullmann U, Winterfeldt E. A short route to cephalostatin analogues. J Chem Soc Perkin Trans 1993;1:2865–7.
[18] Shawakfeh KQ, Al-Said NH, Al- Zoubi RM. Synthesis of bis-diosgenin pyrazine dimers: new cephalostatin analogs. Steroids 2008;73/6:579–84.
[19] Schroepfer JG. Oxysterols: modulators of cholesterol metabolism and other processes. Physiol Rev 2000;80:361–554.
[20] Gregorio-King C, Gough T, Van Der Meer G, Hosking J, Waugh C, McLeod JJ. Mechanisms of resistance to the cytotoxic effects of oxysterols in human leukemic cells. Steroid Biochem Mol Biol 2004;88:311–20.
[21] Blunt JW, Stothers JB. ^{13}C N.M.R spectra of steroids: a survey and commentary. Org Mag Res 1977;9:439–63.
[22] Bogdan AS, Dragana RM, Dosen-Micovic L. Oxidation of steroidal 5-en-3β-ols with Jones reagent in ether. Steroids 1994;59(5):330–4.
[23] Sica D, Musumeci D, Zollo F, De Marino S. Reactivity of steroidal dienes towards the methyltrioxorhenium/H_2O_2-urea oxidation system: isolation and characterization of new oxygenated steroids. Eur J Org Chem 2001: 3731–9.