Side-chain opening of steroidal sapogenins to form 22-oxocholestanic skeletons. An approach to analogues of the aglycone of the potent anticancer agent OSW-1

María A. Fernández-Herrera, Jesús Sandoval-Ramírez,* Socorro Meza-Reyes, and Sara Montiel-Smith

Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla. Ciudad Universitaria, San Manuel, 72570 Puebla, Pue., México. E-mail: jsandova@siu.buap.mx

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Abstract. The side-chain opening of 25R and 25S steroidal sapogenins to form 22-oxocholestanic skeletons is described. The transformation was produced under mild conditions providing high yields (70-87%), in a one-pot procedure (some acetylated starting material is recovered). This methodology yields 17-deoxy-26-oxo analogues of the aglycone of the potent anticancer agent OSW-1. All products were fully characterized by 1D and 2D NMR; the most representative displacements are briefly discussed.

Keywords: steroidal sapogenins, acetylation, aglycone, OSW-1.

Introduction

Steroidal sapogenins are natural products obtained from sapogenins, a group of glycosides widely distributed in plants [1]. In nature, most sapogenins have the 22R configuration, and with regard to C-25 there are two kinds of sapogenins: 25R (the methyl group in C-25 is equatorial oriented), as in diosgenin (1) and hecogenin (2); or 25S (the methyl group in C-25 is axial oriented), as in sarsasapogenin (3, Figure 1). It is very important to highlight the difference of absolute configuration at this center because in many side-chain transformations, products and yields may vary; depending on such configuration.

 Sapogenins are all-important natural compounds because of their use in the preparation of some steroidal biologically active products. In the 40s, sapogenins achieved great economical importance because of their transformation into pregnane derivatives; thus, diosgenin (1) and sarsasapogenin (3) were readily transformed into progesterone [2]; hecogenin (2) was transformed into cortisone and betamethasone [3]. More recently, the steroidal cholestanic saponin OSW-1 (4, Figure 2) and its family analogues (5 to 8) were isolated from the bulbs of Ornithogalum saundersiae by Sashida and coworkers [4].

**Fig. 1.** Structures of natural steroidal sapogenins.

|   | R₁ | R₂ |
|---|---|---|
| 4 | H  | p-methoxybenzoyl (OSW-1) |
| 5 | H  | 3,4-dimethoxybenzoyl |
| 6 | H  | (E)-cinnamoyl |
| 7 | Glc | p-methoxybenzoyl |
| 8 | Glc | (E)-cinnamoyl |

**Fig. 2.** Structure of OSW-1 and its family of cholestane glycosides.
desired protosapogenin 9 (Scheme 1) which represents the ideal pathway for the formation of analogues of the OSW-1 aglycone. However such a system is very reactive under acidic and neutral conditions, displacing instantaneously the equilibrium to the spiroketal moiety. Until now this kind of opening has been a hard challenge.

Scheme 1. Equilibrium between the spiroketal side-chain and the corresponding protosapogenin skeleton.

An initial effort to obtain a protosapogenin side-chain attempting to trap the carbonyl group at C-22, was reported by Djerassi [8]. The treatment of diosgenin acetate 1a with BF3·OEt2 in the presence of ethanedithiol provided the 26-thioacetol 11 instead of the 22-thioketal 10. The intramolecular redox reaction occurred at room temperature in 2 h (Scheme 2); later, similar results were reported by Tian [9].

Scheme 2. Attempted sapogenin side-chain opening in order to obtain a protosapogenin.

Results and Discussion

Following recent work on new transformations of the spiroketal moiety of sapogenins to obtain interesting steroidal structures desirable for partial synthesis [10], an attempt was made to trap the protosapogenin side-chains of 1-3, protecting in situ their diol functionalities (at C-16 and C-26). In this way, cyclization of the cholestanic protosapogenin 9 towards the spirostane skeleton would be avoided. Our studies established the best way to open the E ring to afford 22,26-epoxycholestanic frameworks 12-14, Scheme 3) by means of Ac2O/Lewis acid at room temperature [10a,b]. In this report, a mild opening process of the spiroketal moiety towards the corresponding protected protosapogenin is described. When sapogenins 1-3 were treated by means of Ac2O, BF3·OEt2, in a range of temperatures between 0 and -5 °C, the resulting products were the 22-oxocholestanic-26-acetylated skeletons 15-17, yielded in 70-87% (Table 1). In the range of -5 to 0 °C the 22-oxocholestanic-26-acetylated framework was the main product; however, some starting material was recovered. When the temperature was increased, epoxicholestenes and furostenes were the main products [10a,b].

Under aforementioned conditions, a suitable reaction mechanism is detailed in order to explain the reactivity of the side-chain of sapogenins, (Scheme 3). The oxophilic BF3·OEt2 selectively catalyzes the opening of ring E and the formation of oxonium ion i; simultaneously, the hydroxyl group at C-16 is protected. The intermediate oxonium ion i enables the nucleophilic attack by the acetate anion at C-26 to afford compounds 15-17. At 0 °C we obtained the best yield for compounds 15-17. It is important to notice that the yield of 17 was lower, because of the steric hindrance of the C-27 axial methyl group.

Scheme 3. Plausible mechanism for the formation of 22-oxocholestanic-26-acetylated side-chains.

The transformation of the ketal group at C-22 into the corresponding C-22 ketone produced the expected downfield shifts for both H-23 methylenic and H-20 methynic protons. The latter and the signal pattern for H-26 protons; this evidence allowed the full assignment of such acetates are long distance-coupled with their corresponding 3, 16 and 26 protons; this evidence allowed the full assignment of such signals.

The spectra of compounds 15-17 are displayed in Figure 3; around the region of 5.40 to 2.20 ppm. Towards 5.00 ppm 1H signals appear for all compounds. The signals for H-3 of compounds 15-17 are characterized by HMBC experiment, the carbonyl groups of such acetates are long distance-coupled with their corresponding 3, 16 and 26 protons; this evidence allowed the full assignment of such signals.

Table 1. Influence of temperature in the yield of 15-17.

| Compound | -15 °C | -10 °C | -5 °C | 0 °C | 5 °C | 10 °C | 15 °C |
|----------|-------|-------|-------|------|------|-------|-------|
| 15       | 3     | 20    | 80    | 85   | 72   | 12    | 0     |
| 16       | 2     | 21    | 83    | 87   | 75   | 10    | 0     |
| 17       | 2     | 19    | 61    | 70   | 50   | 8     | 0     |

The transformation of the ketal group at C-22 into the corresponding C-22 ketone produced the expected downfield shifts for both H-23 methylenic and H-20 methynic protons. The latter and the signal pattern for H-26 protons (close δ values) indicate that rings E and F are opened. Table 2 shows the main 1H and 13C chemical shifts observed for compounds 15-17. The acetate methyl groups at positions 3, 16 and 26 were observed. The signals for H-3, 16 and 26 were characterized by HMBC experiment, the carbonyl groups of such acetates are long distance-coupled with their corresponding 3, 16 and 26 protons; this evidence allowed the full assignment of such signals.

The spectra of compounds 15-17 are displayed in Figure 3; around the region of 5.40 to 2.20 ppm. Towards 5.00 ppm 1H signals appear for all compounds. The signals for H-3 in spectra of 15 and 16 are wider than that of 17 because of its 5β stereochemistry, with a typical W½ of 8 Hz. Diastereotopic H-26 signals have the characteristic chemical shifts of an opened cholestanic side-chain, and differ considerably from
those of the starting materials. H-26 protons are displayed as an ABX system.

The full assignments of the $^{13}$C NMR signals of the 22-oxocholestanic products were obtained with the aid of DEPT, HSQC and HMBC experiments. The resonance signals for C-23 and C-20 in all cases were shifted downfield because of the effect of the new carbonyl group at C-22. As predicted in the hypothesis of the reaction mechanism, the C-16β and C-26 hydroxyl groups were protected in situ. The $^{13}$C data showed the δ for C-16 and C-26 as a typical acetate protected alcohol and the corresponding methyl and carbonyl groups of the acetates were fully assigned. Data are summarized in Table 3.

Table 2. Selected $^1$H and $^{13}$C NMR data for compounds 15-17 (δ in ppm, CDCl$_3$).

| Position | Compound | $^1$H (δ in ppm) | $^{13}$C (δ in ppm) |
|----------|----------|------------------|---------------------|
| 3        | 15       | 4.58             | 73.8                |
| 16       | 15       | 4.97             | 75.6                |
| 18       | 15       | 0.87             | 13.3                |
| 19       | 15       | 1.02             | 19.4                |
| 20       | 15       | 2.94             | 43.6                |
| 21       | 15       | 1.14             | 16.9                |
| 22       | 15       | -                | 212.3               |
| 23       | 15-17    | 2.62, 2.26       | 38.3, 38.1          |
| 26       | 15-17    | 3.89             | 68.8, 68.9          |
| 27       | 15-17    | 0.92             | 16.7, 16.6          |
| C=O-Ac-3 | 15-17    | -                | 170.2, 170.5        |
| C=O-Ac-16| 15-17    | -                | 169.4, 169.5        |
| C=O-Ac-26| 15-17    | -                | 170.9, 171.2        |
| Me-Ac-3  | 15-17    | 2.02             | 21.2, 21.4          |
| Me-Ac-16 | 15-17    | 1.95             | 21.0, 21.0          |
| Me-Ac-26 | 15-17    | 2.05             | 20.8, 20.6          |
| C=O-Ac-15| 15-17    | -                | 170.2, 170.6        |
| C=O-Ac-16| 15-17    | -                | 169.4, 169.5        |
| C=O-Ac-26| 15-17    | -                | 170.9, 171.2        |
| Me-Ac-3  | 15-17    | 2.02             | 21.2, 21.4          |
| Me-Ac-16 | 15-17    | 1.95             | 21.0, 21.0          |
| Me-Ac-26 | 15-17    | 2.05             | 20.8, 20.6          |

Fig. 3. Partial $^1$H NMR spectra of compounds 15-17.
Conclusions

In summary, a novel and efficient opening of the spiroke- 
tal moiety of diosgenin, hecogenin and sarsasapogenin is 
reported. We determined that the temperature is one of the 
most important parameters in order to obtain selectively such 
cholestanic frameworks. At low temperatures (-5 to 0 °C), 
17-deoxy-26-oxy analogues of the aglycone of the potent anti-
cancer agent OSW-1 were obtained in high yields under mild 
reaction conditions.

Experimental Section

General procedure for the formation of 15-17. Sapogenin 1-3 
(7 mmol) was dissolved in 20 mL of CH2Cl2 and 10 mL of 
Ac2O (106 mmol) and cooled down to 0 °C; then, 6 mL of 
BF3·OEt2 (48 mmol) were added dropwise. The mixture was 
stirred for 15 min and the resulting syrup was added to 50 mL 
of iced water. The organic phase was washed with saturated 
solution of NaHCO3 (4x50 mL) and dried over Na2SO4, then 
centrated under reduced pressure. The crude was purified 
by chromatography using silica gel, and a mixture of hexanes/ 
ethyl acetate (85:15) as eluent, to afford compounds 
15-17.

Analytical data for compound 15. Colorless solid; mp 146-148 
°C; [α]D = +21.1 ° (c 1.1, CHCl3). 1H NMR (400 MHz, CDCl3 
δ): 5.35 (1H, d, J6,7eq = 5.2 Hz, H-6), 4.97 (1H, m, H-16), 4.58 
(1H, m, H-3), 3.89 (2H, d, J26,25 = 6.4 Hz, H-26), 2.94 (1H, 
dq, J20,21 = 6.8 Hz, J20,17 = 4.0 Hz, H-20), 2.62 (1H, m, H- 
23a), 2.40 (1H, m, H-15a), 2.32 (1H, s, H-16a), 1.95 (3H, s, 
CH3CO2-16), 2.02 (3H, s, CH3CO2-3), 2.05 (3H, s, CH3CO2- 
26), 1.14 (3H, d, J21,20 = 6.8 Hz, CH3-21), 1.02 (3H, s, CH3- 
19), 0.92 (3H, d, J7,27 = 6.8 Hz, CH3-27), 0.87 (3H, s, CH3- 
18). 13C NMR (100 MHz, CDCl3 δ): 36.9 (C-1), 33.4 (C-2), 73.8 
(C-3), 38.1 (C-4), 139.4 (C-5), 122.1 (C-6), 31.6 (C-7), 
31.3 (C-8), 49.8 (C-9), 36.6 (C-10), 20.8 (C-11), 39.6 (C-12), 
41.9 (C-13), 53.9 (C-14), 34.9 (C-15), 75.6 (C-16), 55.0 (C- 
17), 13.3 (C-18), 19.4 (C-19), 43.6 (C-20), 16.9 (C-21), 212.3 
(C-22), 38.3 (C-23), 26.8 (C-24), 32.2 (C-25), 68.8 (C-26), 
16.7 (C-27), 169.4 (CH2CO2-16), 170.2 (CH2CO2-3), 170.9 
(CH3CO2-26), 21.0 (CH2CO2-16), 21.2 (CH2CO2-3), 20.8 
(CH2CO2-26). IR (cm-1): 2936 (CH3), 1728 (C=O, acetate), 
1708 (C=O, ketone), 1708 (C=O, ketone). HRMS for C33H44O7 
Calcd: 558.3557. Found: 558.3551.

Analytical data for compound 17. Colorless syrup; [α]D = +37.8 ° 
(c 0.73, CHCl3). 1H NMR (400 MHz, CDCl3 δ): 5.07 
(1H, s, H-3), 4.97 (1H, m, H-16), 3.90 (2H, m, H-26), 2.94 
(1H, dq, J20,21 = 7.2 Hz, J20,17 = 4.0 Hz, H-20), 2.67 (1H, 
m, 23a), 2.39 (1H, m, H-15a), 2.31 (1H, m, H-23b), 2.05 
(3H, s, CH3CO2-26), 2.03 (3H, s, CH3CO2-26), 2.00 (3H, s, 
CH3CO2-16). IR (cm-1): 2933 (CH), 1722 (C=O, acetate), 1710, 
and 1707 (C=O, ketones). HRMS for C33H46O7 Calcd: 574.3506. 

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