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Practical synthesis of 16,22-diketocholesterol acetate, a precursor of anticancer saponin OSW-1, from diosgenin

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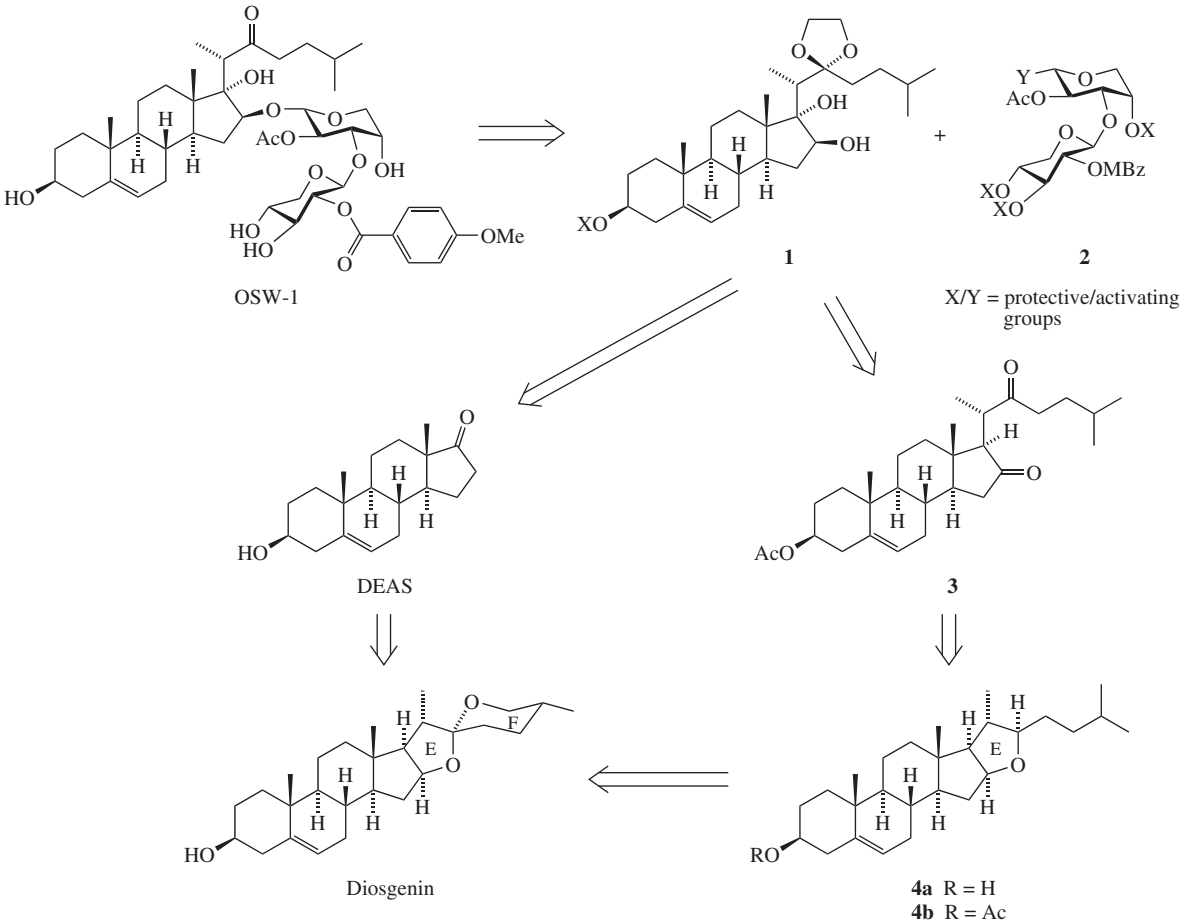
An improved method using dibromide protection of the 5,6-double bond has been developed for the synthesis of the title compound, which is a key intermediate in the synthesis of saponin OSW-1 from diosgenin.

A steroidal glycoside (saponin) known as OSW-1 (Scheme 1) has been isolated in 1992[1] (along with several related structural analogues[2]) from the bulbs of a decorative plant *Ornithogalum saundersiae* (*Liliaceae*). A few years later OSW-1 was found to possess a very high cytostatic activity (IC_{50} 0.02–0.3 nM) towards a wide variety of cancer cells, which is 10 to 100 times more potent than many anticancer agents clinically applied today (mitomycin C, adriamycin, cisplatin, campotecin, taxol, etoposide, *etc.*).[2,3] In the meantime, OSW-1 is less toxic for healthy cells than the agents mentioned above. OSW-1 was also found to possess a considerable anticancer activity *in vivo* when administered in single dose (0.01 mg kg^{-1}). The interest in OSW-1 as a potential new anticancer agent was amplified when its unique cytostatic activity mechanism was revealed, which involves selective structural and functional damaging of mitochondrial membranes resulting in an increase in cytosolic calcium inducing apoptosis.[3]

This information induced intense studies aimed at total synthesis of OSW-1 and its structural analogues. The first synthesis was published in 1999,[4] and it was immediately followed by other syntheses including those of numerous analogues.[5,6] All the syntheses employ a strategy that is conventional for saponins, namely, separate syntheses of a steroidal aglycon and a glycoside fragment in selectively protected forms **1** and **2** (Scheme 1) followed by their conjugation. Commercially available diosgenin and dehydroepiandrosterone (DEAS) were used almost in all the syntheses as the starting steroids. There are two reasons



Scheme 1

why the use of diosgenin as the starting compound[5] appears more expedient than the use of DEAS.[4,6] First, diosgenin has the same cholestane carbon skeleton as OSW-1 and three of the four oxygen functions of aglycon **1**, *i.e.*, the synthesis can be 'atom-economic'. Second, diosgenin is obtained from natural sources as aglycon of several saponins that are abundant in numerous plants of *Dioscoreaceae* species,[7] whereas DEAS is mostly obtained by multistep chemical conversion from the same diosgenin.[8]

The main problem in the use of diosgenin as the starting compound in OSW-1 syntheses is that the 'unneeded' oxygen-containing heterocyclic rings E and F of the spiro ketal moiety have to be opened without involving the C^{20} asymmetric centre, which has an *S*-configuration required for OSW-1. This problem was easily solved[5(a),(c)] for ring F using methods developed previously.[9,10] All these methods involve some kind of reduction at diosgenin C^{22} centre to give pentacyclic steroids **4** with tetrahydrofuran ring E. Opening of the latter, however, constitutes a considerable problem and is the crucial point in the syntheses. Acid-catalysed ring opening proved to be impossible because of cationoid rearrangements of the carbon skeleton.[11] The only efficient way of opening E ring in steroids of type **4** was found to involve vigorous oxidation to give 16,22-diketones **3**. At first this kind of oxidation was performed using 2,2-dimethyldioxirane (DMD),[5(a),(c),(e)] later by a large excess of the $KMnO_4$–$Fe_2(SO_4)_3$ reagent.[12] These methods require several-step protection–deprotection of the 5,6-double bond of compound **4** in the form of *i*-steroid (6-acetoxy-3,5-cyclosteroid) or 5,6-epoxide and are characterised by low substrate conversion or by the need in very laborious preparation of concentrated (0.15 M) DMD solution.[13] A one-stage preparation of diketone **3** by oxidation of steroid **4b** without 5,6-double bond protection with $K_2Cr_2O_7$ in AcOH at 70 °C in 40% yield (57% with respect to non-recovered **4b**) has also been described.[9(b)] In this reaction, a parallel side process involves a considerable oxidation of both the original **4b** and the product **3** at allylic position C^7 to give the corresponding 7-keto derivatives, thus making the procedure poorly reproducible. In our experiments, the average yield of diketone **3** did not exceed 20% after chromatographic isolation.

Our work on the synthesis of OSW-1 from diosgenin required multi-gram amounts of diketone **3**. The drawbacks of the known methods described above forced us to search for a more convenient synthesis of this key intermediate. Since almost all complications in syntheses of diketone **3** originate from the existence in the starting steroids **4** of a 5,6-double bond sensitive to oxidation, this problem might be solved using a more convenient protection of this double bond. To this end, we used classical protection by bromination (Scheme 2).[14,15]

In order to convert steroid **4b** to the corresponding 5,6-dibromide **5**, we applied a rarely used[16] reagent, *viz.*, $KBrO_3$–NaBr in AcOH, which generates Br_2 *in situ*. The convenience of this reagent for our purposes is that it can easily be used in an exactly stoichiometric amount (0.33 and 1.67 mol equivalents of salts, respectively), so that dibromide **5**† that is formed quantitatevely can be employed in the next step without isolation.‡ However, oxidation of this dibromide with $Na_2Cr_2O_7$ in AcOH for 10 h at 70 °C, though it occurs smoothly for ring E, gave a mixture of stereoisomeric 5α,6β- and 5β,6α-dibromodiketones **6**‡ and **7**‡ in a nearly-equilibrium ratio of 1:4. This was very undesired, since of isomers **6** and **7**, only the former can be readily debrominated.

An unpleasant property of dibromide **5**, possessing diaxial positions of both bromine substituents and being a kinetic product of bromination, as well as of other steroidal 5α,6β-dibromides,[15,17] is that they can readily undergo spontaneous isomerization to afford diequatorial (with respect to ring B) 5β,6α-isomers that are more thermodynamically stable. According to ^{1}H NMR data, freshly-prepared dibromide **5** already contains 2–3% of the 5β,6α-isomer,† and the content of the latter increases to 35% after storing its $CDCl_3$ solution for 14 days.§ A similar ability to undergo spontaneous isomerization is also typical of dibromo diketone **6**. Therefore, oxidation of ring E in dibromide **5** had to be performed under significantly milder conditions.

These conditions of oxidation were attained by treatment with a solution of CrO_3 in AcOH for 3 h at 24–26 °C to give dibromo diketone **6** with only a minor admixture of isomer **7**.‡ This oxidation occurs unexpectedly rapidly and results in a complete conversion of the original substrate. An experiment

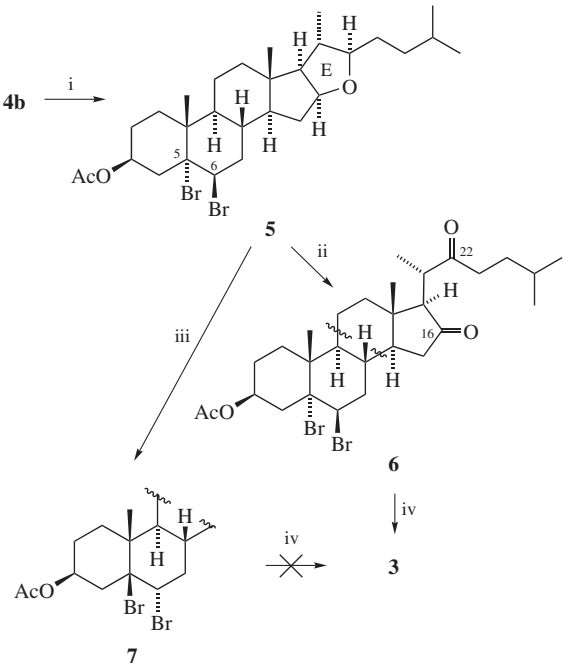
† *(25*R*)-3β-Acetoxy-5α,6β-dibromofurostane* **5**: needles of solvate with 0.5 AcOH molecule (from the reaction mixture) that undergo desolvation on drying *in vacuo* to give a colourless glass. IR (KBr, ν/cm^{-1}): 1740 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.84 (s, 3H, $C^{18}H_3$), 0.88 (d, 6H, $C^{26}H_3$ + $C^{27}H_3$, *J* 6.7 Hz), 1.00 (d, 3H, $C^{21}H_3$, *J* 6.7 Hz), 1.48 (s, 3H, $C^{19}H_3$), 2.05 (s, 3H, OAc), 2.09 (s, 1.5H, AcO*H*), 3.31 (m, 1H, H^{22}), 4.30 (m, 1H, H^{16}), 4.81 (m, 1H, H^6), 5.48 (tt, 1H, H^3, *J* 1.5 and 5.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 16.80, 19.16, 20.23, 21.01, 21.40, 22.58, 22.66, 26.26, 28.33, 30.73, 31.46, 32.10, 35.90, 36.61, 37.38, 38.02, 39.35, 41.19, 42.01, 42.07, 47.43, 55.51, 55.79, 65.32, 72.03, 83.01, 88.07, 90.53, 170.46. MS (ESI), *m/z*: 601.1886/603.1868/605.1847 (M + H)$^+$, 623.1706/625.1688/627.1667 (M + Na)$^+$; 639.1449/641.1401/643.1402 (M + K)$^+$. Calc. for $C_{29}H_{46}Br_2O_3$ $^{79}Br_2$/$^{79}Br^{81}Br$/$^{81}Br_2$ isotopomers: 601.1892/603.1871/605.1851 (M + H); 623.1711/625.1691/627.1670 (M + Na); 639.1451/641.1430/643.1410 (M + K). Found (%): C, 58.17; H, 7.86 (n = 0); Br, 24.94 (n = 0.5). Calc. for $C_{29}H_{46}Br_2O_3 \cdot n$AcOH (%): C, 57.81; H, 7.70 (n = 0); Br, 25.27 (n = 0.5).

5β,6α-Isomer of dibromide **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.78 (s, 3H, $C^{18}H_3$), 0.88 (d, 6H, $C^{26}H_3$, $C^{27}H_3$, *J* 6.7 Hz), 0.99 (d, 3H, $C^{21}H_3$, *J* 6.7 Hz), 1.25 (s, 3H, $C^{19}H_3$), 2.07 (s, 3H, OAc), 2.36 (dd, 1H, C^4*H*H, *J* 4.2 and 16.8 Hz), 2.87 (br. d, 1H, C^4H*H*, *J* 16.8 Hz), 3.31 (m, 1H, H^{22}), 4.30 (m, 1H, H^{16}), 4.87 (dd, 1H, H^6, *J* 5.0 and 12.6 Hz), 5.15 (br. s, 1H, H^3).

‡ *3β-Acetoxy-5α,6β-dibromocholestane-16,22-dione* **6**. Water (1 ml) was added to a solution of steroid **4b** (1.00 g, 2.26 mmol) and a suspension of NaBr (388 mg, 3.77 mmol) in AcOH (20 ml) at 26 °C. Then, $KBrO_3$ (126 mg, 0.75 mmol) was added with stirring in one portion to the resulting fine suspension of **4b** in a solution of NaBr. A thick suspension of dibromide **5** crystals was formed in 1 h (the compound can be isolated by dilution with water). The suspension was diluted with AcOH (20 ml) to give a solution; a 1.0 M CrO_3 solution in 80% AcOH (15.8 ml) was added, and the mixture was stirred at 24–26 °C for 3 h (until disappearance of **5** according to TLC). The reaction mixture was slowly diluted with water (250 ml); the light-yellow powder formed was filtered off, washed with water and dissolved in CH_2Cl_2. The solution was dried with $MgSO_4$, filtered through ~1 g of silica gel, and evaporated to dryness to afford 1.38 g (99%) of dibromo diketone **6** as a light-yellow glass. IR (KBr, ν/cm^{-1}): 1716, 1736 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.83 (s, 3H, $C^{18}H_3$), 0.92 (d, 6H, $C^{26}H_3$, $C^{27}H_3$, *J* 6.0 Hz), 1.05 (d, 3H, $C^{21}H_3$, *J* 6.1 Hz), 1.50 (s, 3H, $C^{19}H_3$), 2.05 (s, 3H, OAc), 4.83 (m, 1H, H^6), 5.49 (tt, 1H, H^3, *J* 5.4 and 10.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.40, 15.44, 20.14, 20.84, 21.37, 22.52 (2C), 26.16, 27.12, 30.09, 32.28, 36.29, 37.03, 37.29, 38.52, 40.46, 41.88, 41.98, 42.08, 43.39, 47.04, 49.90, 55.27, 66.14, 71.88, 87.15, 170.41, 213.75, 217.05. MS (ESI), *m/z*: 617.1611 (M + H)$^+$, 639.1423 (M + Na)$^+$, 655.1213 (M + K)$^+$. Calc. for $C_{29}H_{46}{}^{79}Br^{81}BrO_3$: 617.1664 (M + H), 639.1483 (M + Na), 655.1223 (M + K).

3β-Acetoxy-5β,6α-dibromocholestane-16,22-dione **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.78 (s, 3H, $C^{18}H_3$), 0.91 (d, 6H, $C^{26}H_3$, $C^{27}H_3$, *J* 5.8 Hz), 1.04 (br. s, 3H, $C^{21}H_3$), 1.27 (s, 3H, $C^{19}H_3$), 2.08 (s, 3H, OAc), 4.83 (m, 1H, H^6), 5.19 (br. s, 1H, H^3).

§ It was shown that isomerization of 3β-acetoxy-5α,6β-dibromoandrostan-17-one (5α,6β-Br_2-AS) to the corresponding 5β,6α-dibromide occurs even faster in more polar solvents and is accompanied by debromination with the solvent into the corresponding 5,6-olefin (Δ^5-AS). Keeping solutions of 5α,6β-Br_2-AS for 16 days at room temperature results in 5α,6β-Br_2-AS:5β,6α-Br_2-AS:Δ^5-AS mixtures with ratios of 70:22:8 ($CHCl_3$), 38:14:48 (Me_2CO), 32:37:31 (MeCN). It is evident that the rate of debromination depends both on the rate of isomerization and on the expected facility of solvent bromination with nascent bromine.



Scheme 2 *Reagents and conditions*: i, NaBr, $KBrO_3$, AcOH, 26 °C, 1 h; ii, CrO_3, AcOH, 24–26 °C, 3 h, 99% (from **4b**); iii, $Na_2Cr_2O_7$, AcOH, 70 °C, 5 h, 72%; iv, NaI, Na_2SO_3, Me_2CO–H_2O, 25 °C, 1 h, 60%.

on oxidation of a 1:1 mixture of dibromide **5** and the corresponding olefin **4b** under these conditions revealed that the dibromide reacted 3–5 times faster than the olefin. Most likely, the two bromine atoms in the ring B in dibromide **5** activate through an unknown mechanism the tetrahydrofuran ring E in the same molecule for oxidation.

Dibromo diketone **6** should be used in the next stage without purification or prolonged storage. Its debromination into the target diketone **3** readily occurs on treatment with a NaI solution in acetone. The admixture of isomer **7** remains unchanged under these conditions and it is inexpedient to recover it.¶ The overall yield of diketone **3** from pentacyclic steroid **4b** reaches 60%, while a major fraction of the product (48%) can be isolated by direct crystallisation without the use of chromatography.††

The two-stage method that we have developed employs no laborious procedures and provides a relatively high yield of diketone **3** without recyclisation of the recoverable materials thus being a preparative technique. This method increases considerably the efficiency of the published syntheses[5] of anticancer saponin OSW-1 from diosgenin.

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¶ According to literature data,[17] steroidal 5β,6α-dibromides do not react with NaI in acetone. Nevertheless, we have found that 5β,6α-dibromodiketone **7** can be debrominated by ~80% on treatment with a NaI solution in Me_2CO (50 °C, 10 h) or in MeCN (70 °C, 4 h), but the yields of diketone **3** are low, possibly due to partial isomerization at C^{20} under these drastic conditions.[18]

†† *3β-Acetoxycholest-5-ene-16,22-dione* **3**. A solution of $NaI·2H_2O$ (3.13 g, 16.8 mmol) in water (6.2 ml) and then a solution of Na_2SO_3 (2.12 g, 16.8 mmol) and AcOH (1.0 ml, 16.8 mmol) in water (10.6 ml) were added to a solution of crude dibromo diketone **6** (2.07 g, 3.36 mmol) in acetone (62 ml) at 25 °C with vigorous stirring. The double-layered mixture was stirred for 1 h, diluted with water (200 ml), and acetone was evaporated *in vacuo*. The transparent supernatant was decanted off from the semicrystalline precipitate, which was washed with water, dried and dissolved in CH_2Cl_2; the solution was filtered through ~1 g of silica gel and evaporated to dryness. Crystallization of the light-yellow precipitate from MeOH gave 741 mg (48%) of diketone **3**, mp 155–156 °C (lit.,[9(b)] mp 151.5–152.5 °C; lit.,[13] 152–154 °C); the ^{1}H NMR spectrum was identical to that reported elsewhere.[9(b),13] Chromatography on silica gel allowed another 182 mg (12%) of crystalline diketone **3** to be isolated; the overall yield is 60%.

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