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Mechanistic insights on the reactivity of furospirostanes with the 16β,22:22,25-diepoxy-23-acetoxymethyl-24-methyl side chain

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ABSTRACT

F-ring opening in spirostanes with the 16β,22:22,25-diepoxy-23-acetoxymethyl-24-methyl side chain produces a Δ^{22}-intermediate with an allylic acetoxy group. For this reason the reactivity profile of these compounds deviates from that observed in other naturally occurring or synthetic spirostanes and furospirostanes

1. Introduction

The spiroketal assemblies (see Fig. 1) are present in widespread compounds that can be isolated from many marine and terrestrial organisms: plants, fungi and insects among others. The vast number and the growing pharmacological importance of compounds containing spiroketal assemblies have triggered increasing interest in both their synthesis and studies of their chemical reactivity [1–5]. In addition to the spirostanic sapogenins which bear the 1,6-dioxaspiro[4,5] decane moiety, furospirostanes, an emerging family of steroids bearing the 16β,22:22,25-diepoxy moiety in the chain, that are considered 1,6-dioxaspiro[4.4] nonane derivatives are attracting increasing attention.

The increasing number of naturally occurring bioactive steroids that bear the furospirostane side chain includes compounds that have shown interesting antitumor activity such as ritterazines **(1)**, cephalostatins **(2)** [6–10], hippuristanols **(3)** [11–15], as well as the antihypertensive glycosides of nuatigenin **(4)** [16] among others (see Fig. 2).

Unlike spirostanic sapogenins, the side-chain reactivity of which has received considerable attention in the past 70 years selected Ref. [17–37], the studies of the reactivity of the furospirostane side chain are rather scarce [38–42]. In particular, Fuchs and coworkers hypothesized that the cytotoxic activity of cephalostatins may be related to the possibility of the generation of oxacarbenium ions around C-22 [43–44]. This fact, added to the increasing number of cytotoxic steroids bearing the furospirostane side chain that have been identified, prompts the exploration of the reactivity of this moiety.

As a part of our ongoing program directed to the synthesis of potentially bioactive spiroketals derived from rearranged steroid sapogenins, we envisaged the bromination at C-23 of our previously described furospirostane sapogenin **23S-5** [45] as a possible route to the introduction of additional functionality in the F ring (see Scheme 1).

In the course of the bromination experiments employing pyridine hydrobromide perbromide ($PyrBr_2{\cdot}HBr$) we found that, in addition to the desired bromination at C-23, an unexpected substitution of the C-23′ acetoxy moiety also took place. This led us to explore the particular reactivity of this structural fragment. Herein we describe our findings on the reactivity of the 16β,22:22,25-diepoxy-23-acetoxymethyl-24-methyl side chain.

2. Experimental

Reactions were monitored by TLC on ALUGRAM® SIL G/UV254 plates from MACHEREY–NAGEL. Chromatographic plates were sprayed with a 1% solution of vanillin in 50% $HClO_4$ and heated until color developed. Purifications and separations were performed in pressurized chromatographic columns packed with MACHEREY–NAGEL silica gel 60 (230–400 mesh ASTM). Melting points were measured on a Melt-Temp II apparatus. Mass spectra were registered in a Thermo-Electron spectrometer model DFS (Double Focus Sector). NMR spectra were recorded in $CDCl_3$ solution in a Varian INOVA 400 spectrometer using the solvent signal

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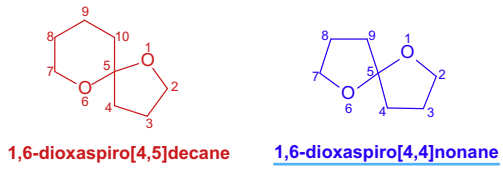


Fig. 1. Common dioxaspiro ketal moieties.

7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C as references. NMR signals assignments were made with the aid of DEPT and a combination of 2D homonuclear (^{1}H–^{1}H) and heteronuclear (^{1}H–^{13}C) correlation techniques, which included ^{1}H–^{1}H COSY, ^{1}H–^{1}H Nuclear Overhauser Effect Spectroscopy (NOESY), and Heteronuclear Single Quantum Correlation (HSQC) [46]. All 2D NMR spectra were recorded using the standard pulse sequences and parameters recommended by the manufacturer.

3. Bromination of 23*S*-5 with $PyH^{+}Br_{3}^{-}$

$PyH^{+}Br_{3}^{-}$ (1.28 g, 3.6 mmol) was added to a solution of **23*S*-5** (518 mg, 1.0 mmol) [45] in acetic acid (25 mL). The mixture was stirred for 1 h at 50 °C and poured into ice/water. The produced solid was filtered off washed with water and dissolved in CH_2Cl_2. The organic solution was dried (anh. Na_2SO_4) and evaporated to produce a mixture of **23*R*-6, 23*S*-6, 23*R*-7** and **23*S*-7** that were separated in a pressurized chromatographic column packed with silica gel (18.5 g) using hexane/ethyl acetate 15:1 as eluent. Order of elution **23*R*-7**, **23*S*-7, 23*S*-6, 23*R*-6**.

(22*S*,23*R*,24*S*)-16β,22:22,25-diepoxy-23-bromo-23-hydroxymethyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol diacetate **(23*R*-6)**. Yield 391.7 mg (0.658 mmol, 65.8%). Mp. 262–264 °C (*from ethyl acetate/hexane*). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.71 (m, 1H, H-3), 4.44 (ddd, J = 8.6, 7.6, 6.1 Hz, 1H, H-16), 4.35 (d, J = 11.8 Hz, 1H, H-23′a), 4.23 (d, J = 11.8 Hz, 1H, H-23′b), 4.06 (dd, J = 8.1 Hz, 1H, H-25 *Pro-S*), 3.55 (dd, J = 8.2, 7.1 Hz, 1H, H-25 *Pro-R*), 2.44 (dq, J = 6.7, 6.7 Hz, 1H, H-20), 2.29 (m, 1H, H-24), 2.11 (s, 3H, CH_3COO-23′), 2.01 (s, 3H, CH_3COO-3), 1.18 (d, J = 6.9 Hz, 3H, H-24′), 1.02 (d, J = 6.9 Hz, 3H, H-21), 0.94 (s, 3H, H.19), 0.88 (s, 3H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.2, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.5, C-8 35.4, C-9 40.5, C-10 34.7, C-11 20.5, C-12 40.1, C-13 41.6, C-14 56.2, C-15 31.7, C-16 81.0, C-17 63.3, C-18 16.4, C-19 23.3, C-20 35.5, C-21 16.3, C-22 117.4, C-23 73.4, C-23′ 69.2, C-24 38.1, C-24′ 19.0, C-25 70.7, CH_3COO-23′ 170.5, CH_3COO-23′ 20.8, CH_3COO-3 170.6, CH_3COO-3 21.4. MS (EI) 515 [M^{+}–Br], 454, 426, 394, 366, 344, 329, 284, 269, 255, 215, 161, 147, 137 (100%). HRMS (EI) *observed* 515.3379 [M^{+}–Br]; *required for* $C_{31}H_{47}O_6$ 515.3372.

(22*S*,23*S*,24*S*)-16β,22:22,25-diepoxy-23-bromo-23-hydroxymethyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol diacetate **(23*S*-6)**. Yield 53.4 mg, (0.09 mmol, 9.0%). Mp. 266–267 °C (*from*

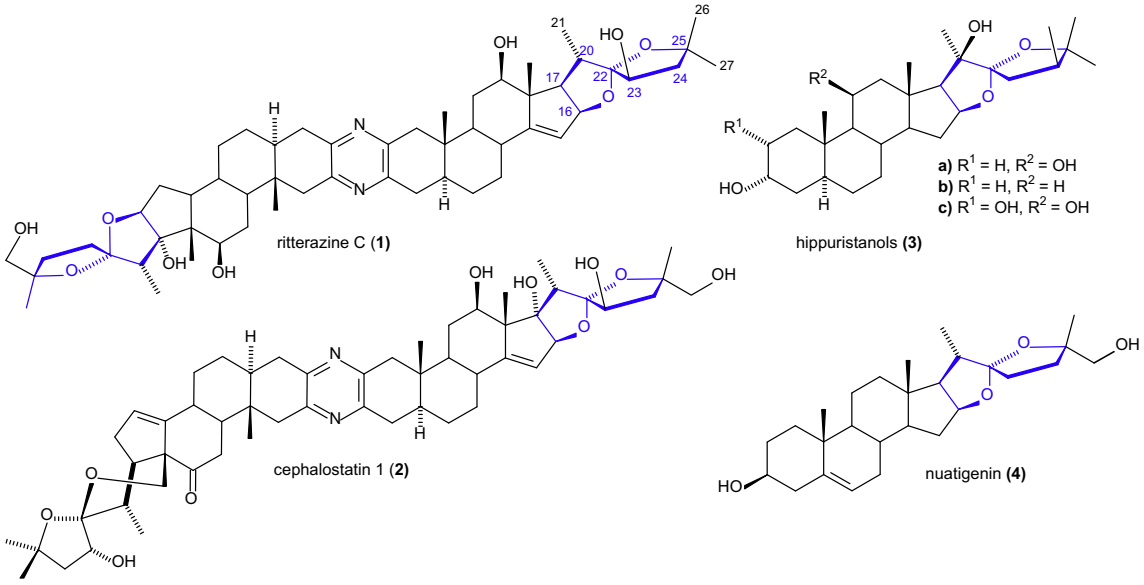
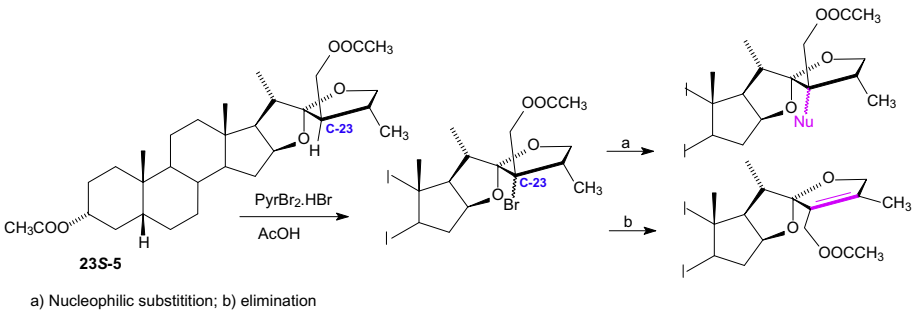


Fig. 2. Some naturally occurring furospirostanes.



Scheme 1. Functionalization of the furospirostane side chain.

ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.71 (m, 1H, H-3), 4.47 (m, 1H, H-16), 4.47 (s, 2H, H-23′), 4.31 (dd, J = 7.9 Hz, 1H, H-25 *Pro-S*), 3.57 (dd, J = 8.2, 3.9 Hz, 1H, H-25 *Pro-R*), 2.89 (m, 1H, H-24), 2.52 (m, 1H, H-20), 2.13 (s, 3H, CH_3COO-23′), 2.02 (s, 3H, CH_3COO-3), 1.26 (d, J = 6.9 Hz, 3H, H-21), 1.16 (d, J = 7.3 Hz, 3H, H-24′), 0.93 (s, 3H, H-19), 0.81 (s, 3H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): C-1 35.0, C-2 26.5, C-3 74.2, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.5, C-8 35.4, C-9 40.5, C-10 34.7, C-11 20.4, C-12 39.7, C-13 41.3, C-14 56.3, C-15 31.9, C-16 81.6, C-17 63.9, C-18 16.4, C-19 23.3, C-20 39.4, C-21 18.2, C-22 119.1, C-23 73.8, C-23′ 65.9, C-24 46.5, C-24′ 16.7, C-25 72.6, CH_3-*C*OO-3 170.6, CH_3COO-23′ 170.6, *C*H_3COO-3 21.5, *C*H_3COO-23′ 21.0. MS (EI) [M^+−Br], 454, 426, 329, 315, 284, 269, 255, 215, 159, 147, 137 (100%). HRMS (EI) *observed* 515.3377 [M^+−Br]; *required for* $C_{31}H_{47}O_6$ 515.3372.

Crystals of **23*R*-6** and **23*S*-6** mounted on glass fiber were studied with Oxford Diffraction Gemini "A" diffractometer with a CCD area detector ($\lambda_{cuk\alpha}$ = 1.54184 Å, monochromator: graphite) source equipped with a sealed tube X-ray source. Unit cell constants were determined with a set of 15/3 narrow frame/runs (1° in ω) scans. Data sets consisted of 1154 and 684 frames of intensity data collected with a frame width of 1° in ω for **23*R*-6** and **23*S*-6** respectively with a crystal-to-detector distance of 55.00 mm. The double pass method of scanning was used to exclude any noise. The collected frames were integrated by using an orientation matrix determined from the narrow frame scans. CrysAlisPro and CrysAlis RED software packages [47] were used for data collection and data integration. Structure solution and refinement were carried out with the programs SHELXS97 and SHELXL97 [48]. For molecular graphics, ORTEP-3 for Windows was employed, and the software used to prepare material for publication was WinGX [49].

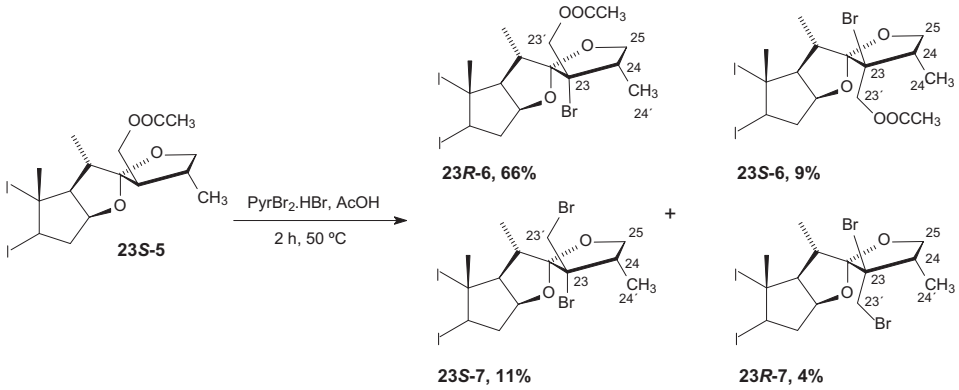
Full-matrix least-squares refinement was carried out by minimizing $(Fo^2 - Fc^2)^2$. All non-hydrogen atoms were refined anisotropically. H atoms attached to C atoms were placed in geometrically idealized positions and refined as riding on their parent atoms, with C–H = 0.96–0.99 Å with U_{iso}(H) = 1.2 U_{eq}(C) for methine and methylene groups, and U_{iso}(H) = 1.5 U_{eq}(C) for methyl group. Crystal data and experimental details of the structure determination are listed in Table 1 [50].

(22*S*,23*R*,24*S*)-16β,22:22,25-diepoxy-23-bromo-23-bromomethyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate **(23*R*-7)**. Yield 24.3 mg, (0.039 mmol, 3.9%). Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.71 (m, 1H, H-3), 4.48 (ddd, J = 7.8, 7.8, 5.1 Hz, 1H, H-16), 4.36 (dd, J = 8.1, 7.1 Hz, 1H, H-25 *Pro-S*), 4.03 (d, J = 11.6 Hz, 1H, 23′a), 3.70 (d, J = 11.6 Hz, 1H, 23′b), 3.66 (dd, J = 8.2, 2.4 Hz, 1H, H-25 *Pro-R*), 2.80 (qdd, J = 7.2, 7.2, 2.3 Hz, 1H, H-24), 2.42 (m, 1H, H-20), 2.01 (s, 3H, CH_3COO–), 1.28 (d, J = 7.3 Hz, 3H, H-24′), 1.27 (d, J = 6.8 Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.77 (s, 3H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.2, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.5, C-8 35.5, C-9 40.5, C-10 34.7, C-11 20.4, C-12 39.6, C-13 41.5, C-14 56.3, C-15 32.0, C-16 81.6, C-17 63.8, C-18 16.3, C-19 23.3, C-20 39.4, C-21 18.7, C-22 119.3, C-23 77.3, C-23′ 38.1, C-24 46.6, C-24′ 17.0, C-25 73.2, CH_3COO-3 170.6, *C*H_3COO-3 21.4. MS (EI) 535 [$^{79}Br_2M^+-{}^{79}Br$], 456, 426, 344, 329, 284, 269, 255, 227, 215, 175, 149, 137 (100%). HRMS (EI) *observed* 535.2415 [$^{79}Br_2M^+-{}^{79}Br$] *required for* $C_{29}H_{44}{}^{79}BrO_4$ 535.2423.

(22*S*,23*S*,24*S*)-16β,22:22,25-diepoxy-23-bromo-23-bromomethyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate **(23*S*-7)** yield 66.0 mg, (0.107 mmol, 10.7%).Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 4.72 (m, 1H, H-3), 4.44 (m, 1H, H-16), 4.08 (dd, J = 8.2, 8.2 Hz, 1H, H-25 *Pro-S*), 3.88 (d, J = 10.8 Hz, 1H, H-23′a), 3.75 (d, J = 10.8 Hz, 1H, H-23′b), 3.54 (dd, J = 8.2, 6.8 Hz, 1H, H-25 *Pro-R*), 2.53 (m, 1H, H-24), 2.47 (m, 1H, H-20), 2.02 (s, 3H, CH_3COO), 1.18 (d, J = 6.8 Hz, 3H, H-21), 1.06 (d, J = 6.9 Hz, 3H, H-24′), 0.95 (s, 1H, H-19), 0.92 (s, 3H, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.3, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.5, C-8 35.5, C-9 40.5, C-10 34.7, C-11 20.5, C-12 40.1, C-13 41.7, C-14 56.2, C-15 31.7, C-16 81.1, C-17 63.5, C-18 16.42, C-19 23.3, C- 20 35.4, C-21 16.8, C-22 117.5, C-23 75.6, C-23′ 43.9, C-24 39.5, C-24′ 19.7, C-25 70.4, CH_3COO-3 170.6, CH_3-COO-3 21.5. MS (EI) 535 [$^{79}Br_2M^+-{}^{79}Br$], 456, 426, 344, 329, 315, 269, 255, 159, 149, 137 (100%). HRMS (EI) *observed* 535.2413 [$^{79}Br_2M^+-{}^{79}Br$] *required for* $C_{29}H_{44}{}^{79}BrO_4$ 535.2423.

Table 1
Crystal data and structure refinement for **23*R*-6** and **23*S*-6**.

	23*R*-6	**23*S*-6**
Empirical formula	$C_{31}H_{47}BrO_6$	$C_{31}H_{47}BrO_6$
Formula weight	595.60	595.60
Temperature (K)	298(2) K	298(2) K
Wavelength (Å)	1.54184	1.54184 Å
Crystal system	Monoclinic	Monoclinic
Space group	P21	P21
Unit cell dimensions (Å)	a = 11.0378(4)	a = 13.2911(4)
	b = 7.8265(3)	b = 7.7902(2)
	c = 17.8645(6)	c = 14.5238(3)
	β = 104.658(4)	β = 98.487(2)
Volume (Å^3)	1493.04(9)	1487.33(7)
Z	2	2
Crystal size (mm^3)	0.2171 × 0.1503 × 0.0478	0.4685 × 0.257 × 0.0418
Density (calculated) (Mg/m^3)	1.325	1.330
Absorption coefficient (mm^{-1})	2.202	2.211
F(000)	632	632
Theta range for data collection	4.14°–68.11°	4.21°–68.07°
Index ranges	$-11 \leqslant h \leqslant 13$, $-8 \leqslant k \leqslant 9$, $-20 \leqslant l \leqslant 21$	$-10 \leqslant h \leqslant 15$, $-7 \leqslant k \leqslant 9$, $-17 \leqslant l \leqslant 17$
Reflections collected	10014	5346
Independent reflections	4696 [R(int) = 0.0451]	3737 [R(int) = 0.0220]
Completeness to theta = 66.50°	100.0%	94.6%
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	4696/1/349	3737/1/349
Goodness-of-fit on F^2	0.964	1.103
Final R indices [I > 2sigma(I)]	$R1$ = 0.0405, w$R2$ = 0.0939	$R1$ = 0.0369, w$R2$ = 0.0870
R indices (all data)	$R1$ = 0.0551, w$R2$ = 0.1004	$R1$ = 0.0455, w$R2$ = 0.0919
Largest diff. peak and hole	0.299 and −0.255 e.Å^{-3}	0.485 and −0.453 e.Å^{-3}



Scheme 2. Bromination of the furospirostane side chain.

4. Reaction of 23*S*-5 with HBr in acetic acid

The diacetate **23*S*-5** (258.4 mg, 0.5 mmol) was added to a 33 wt.% solution of HBr in acetic acid (5 mL, 27.2 mmol of HBr). The mixture was stirred for 45 min., poured into ice/water and extracted with ethyl acetate (40 mL). The organic layer was washed with 5% aqueous Na_2CO_3 (4 × 25 mL) and water (2 × 25 mL), dried (anh. Na_2SO_4) and evaporated to produce a mixture of **23*R*-8** and **23*S*-8** that were separated in pressurized chromatographic column packed with silica gel (12 g) using hexane/ethyl acetate 40:1 as eluent. Order of elution **23*S*-8**, **23*R*-8**.

(22*S*, 23*R*, 24*R*)-16β,22:22,25-diepoxy-23′-bromo-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate **(23*R*-8)** yield 41.5 mg, (0.077 mmol, 15.4%). Yellow oil. ^{1}H NMR (400 MHz, *CDCl*$_3$, δ ppm): 4.72 (m, 1H, H-3), 4.41 (ddd, *J* = 8.5, 7.6, 6.0 Hz, 1H, H-16), 3.97 (dd, *J* = 8.0 Hz, 1H, H-25 *Pro-S*), 3.57 (dd, *J* = 10.1, 6.4 Hz, 1H, H-23′a), 3.43 (dd, *J* = 9.2, 8.3 Hz, 1H, H-25 *Pro-R*), 3.34 (dd, *J* = 10.1, 8.3 Hz, 1H, H-23′b), 2.36 (m, *J* = 13.4, 6.8 Hz, 1H, H-20), 2.27 (m, 1H, H-23), 2.02 (s, 3H, CH_3COO-3), 1.13 (d, *J* = 6.7 Hz, 3H, H-24′), 1.00 (d, *J* = 6.9 Hz, 3H, H-21), 0.95 (s, 3H, H-19), 0.82 (s, 3H, H-18). ^{13}C NMR (75 MHz, *CDCl*$_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.3, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.5, C-8 35.4, C-9 40.5, C-10 34.7, C-11 20.6, C-12 40.1, C-13 41.2, C-14 56.2, C-15 31.8, C-16 81.0, C-17 62.9, C-18 16.6, C-19 23.3, C-20 36.1, C-21 16.2, C-22 119.5, C-23 55.7, C-23′ 33.5, C-24 39.9, C-24′ 17.2, C-25 71.4, CH_3COO-3 170.6, *C*H_3COO-3 21.5. MS (FAB) MH^+ 539, MH^+ 537, 459, 457, 255, 147, 133, 91, 83, 73, 57 (100%). HRMS (FAB) *observed* 537.2500 [MH^+] *required for* $C_{29}H_{46}{}^{79}BrO_4$ 537.2579.

(22*S*, 23*S*, 24*R*)-16β,22:22,25-diepoxy-23′-bromo-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate **(23*S*-8)** yield 40.6 mg, (0.076 mmol, 15.2%). Yellow oil. ^{1}H NMR (400 MHz, *CDCl*$_3$ δ ppm): 4.71 (m, 1H, H-3), 4.44 (m, 1H, H-16), 3.95 (dd, *J* = 8.3, 6.4 Hz, 1H, H-25 *Pro-S*), 3.63 (dd, *J* = 8.3, 2.7 Hz, 1H, H-25 *Pro-R*), 3.44 (m, 2H, H-23′), 2.47 (m, 1H, H-24), 2.47 (m, 1H, H-23), 2.14 (m, 1H, H-20), 2.02 (s, 3H, CH_3COO-3), 1.13 (d, *J* = 6.8 Hz, 3H, H-24′), 0.97 (d, *J* = 6.9 Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.75 (s, 3H, H-18). ^{13}C NMR (75 MHz, *CDCl*$_3$ δ ppm): C-1 35.0, C-2 26.6, C-3 74.3, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.6, C-8 35.3, C-9 40.5, C-10 34.7, C-11 20.6, C-12 40.1, C-13 41.0, C-14 56.1, C-15 31.8, C-16 81.7, C-17 61.1, C-18 16.6, C-19 23.3, C-20 38.2, C-21 14.4, C-22 118.3, C-23 48.9, C-23′ 29.2, C-24 34.0, C-24′ 14.3, C-25 73.9, CH_3COO-3 170.6, *C*H_3COO-3 21.5. MS (FAB) MH^+ 539, MH^+ 537, 457, 455, 397, 315, 255, 147, 133, 107, 95, 91, 69, 57, 55 (100%).

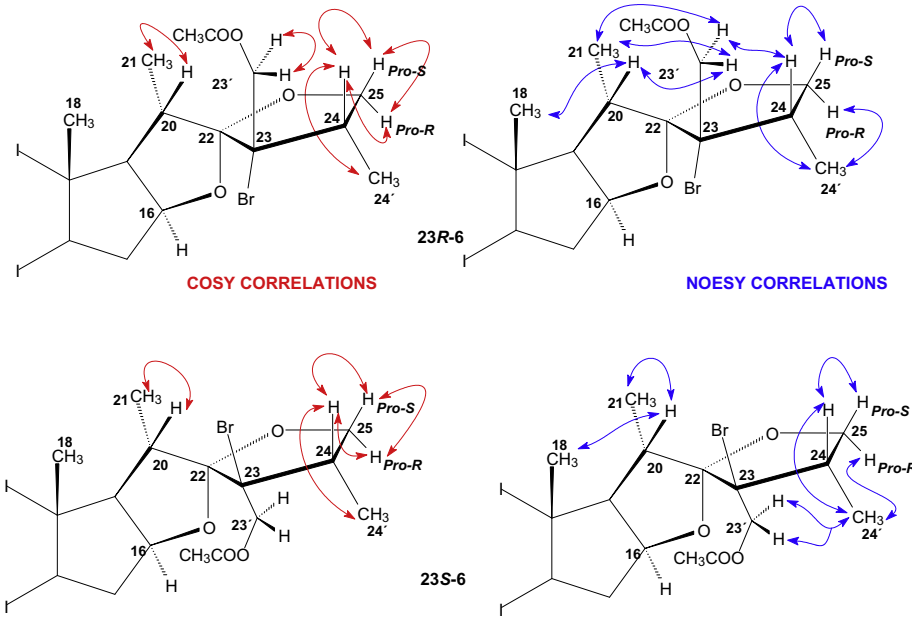


Fig. 3. Selected COSY and NOESY correlation observed in compounds **23*R*-6** (upper) and **23*S*-6** (lower).

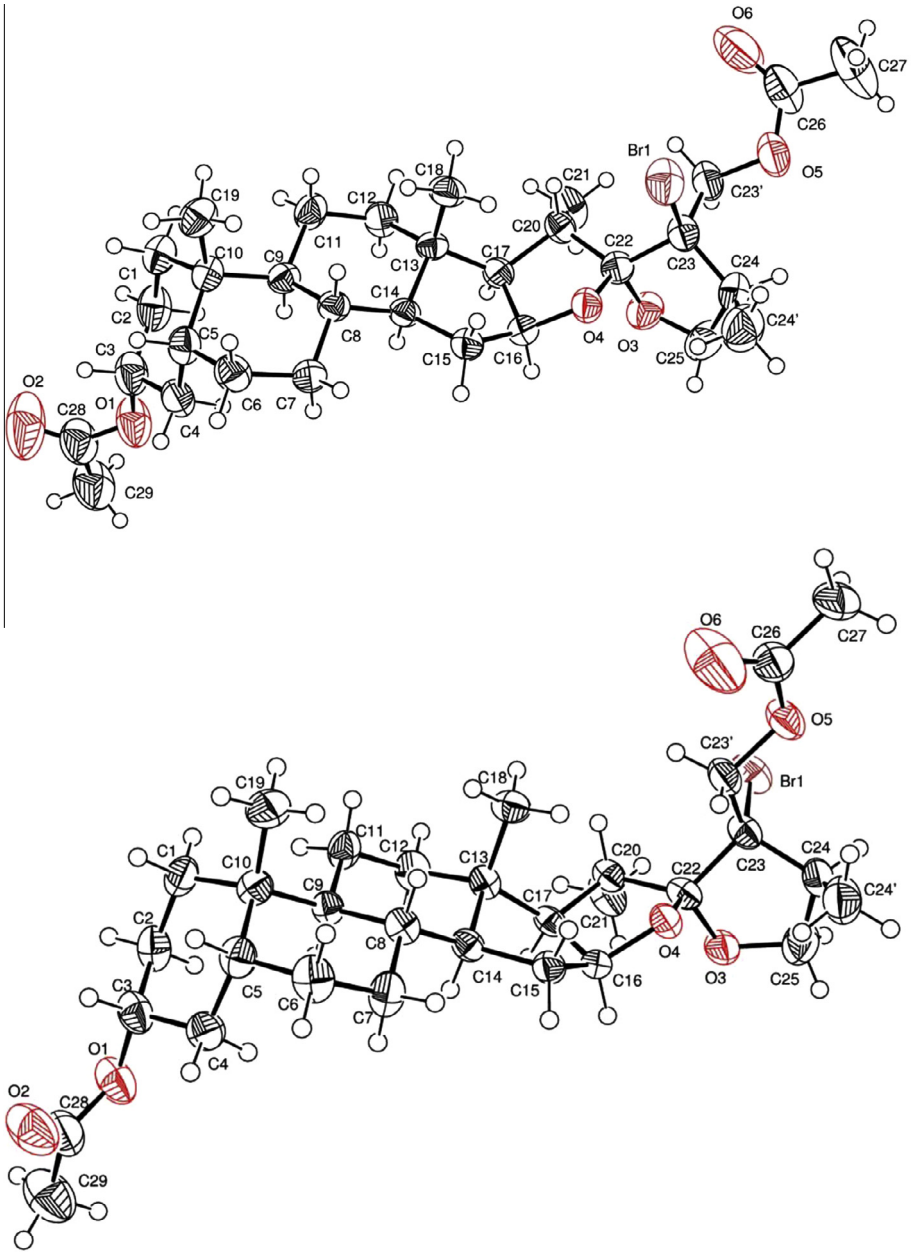


Fig. 4. Crystal structures of **23*R*-6** (upper) and **23*S*-6** (lower) with the thermal ellipsoids drawn at 50% of probability.

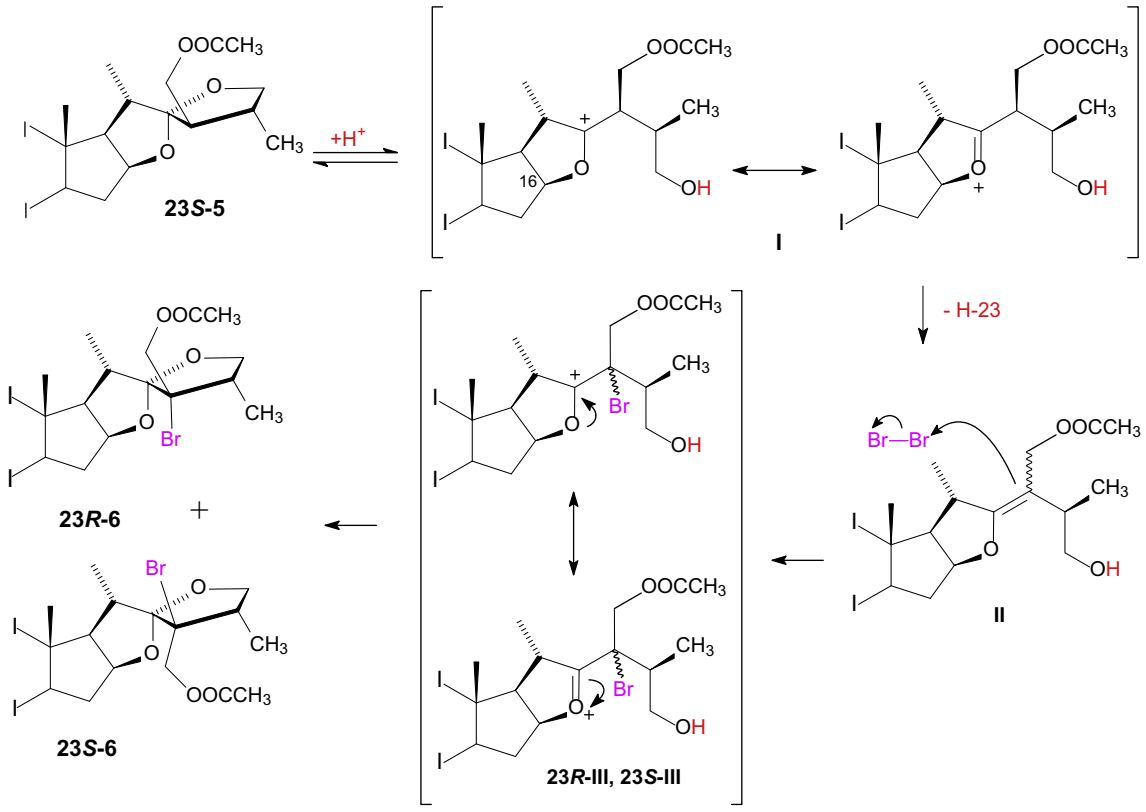
HRMS (FAB) *observed* 537.2495 [MH^+] *required for* $C_{29}H_{46}{}^{79}BrO_4$ 537.2579.

5. Reaction of 23*S*-5 with acetic acid and $BF_3{\cdot}Et_2O$

$BF_3{\cdot}Et_2O$ (1.5 mL, 12.2 mmol) was added to a solution of **23*S*-5** (518 mg, 1.0 mmol) in acetic acid and the mixture was stirred for 5 h before slow addition of 10% aqueous $NaHCO_3$ solution (20 mL) and extraction with ethyl acetate (25 mL). The organic layer was washed with 10% aqueous $NaHCO_3$ solution (8 × 20 mL) water (3 × 20 mL), dried (anh. Na_2SO_4) and evaporated to afford a mixture of the starting material, **23*S*-5**, the epimeric **23*R*-5**, and olefin **9** that were separated in pressurized chromatographic column packed with silica gel (15 g) using hexane/ethyl acetate 97:3 as eluent. Order of elution **9**, **23*R*-5**, **23*S*-5**.

(22*S*, 23*R*, 24*R*)-16β,22:22,25-diepoxy-23-hydroxymethyl-24-methyl-26,27-dinor-5β-choles-tan-3α-ol diacetate **(23*R*-5)**. Yield 86.1 mg, (0.167 mmol, 16.7%). Yellow oil. ^{1}H NMR (400 MHz, *CDCl*$_3$, δ ppm): 4.72 (m, 1H, H-3), 4.45 (m, 1H, H-16), 4.18 (d, *J* = 6.5 Hz, 2H, H-23′), 3.95 (dd, *J* = 8.2, 6.7 Hz, 1H, H-25 *Pro-S*), 3.56 (dd, *J* = 8.3, 3.6 Hz, 1H, H-25 *Pro-R*), 2.38 (m, 1H, H-23), 2.38 (m, 1H, H-24), 2.23 (dq, *J* = 6.9. 6.9 Hz, 1H, H-20), 2.04 (s, 3H, CH_3COO-23′), 2.02 (s, 3H, CH_3COO-3), 1.05 (d, *J* = 6.7 Hz, 3H, H-24′), 0.97 (d, *J* = 6.9 Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.76 (s, 3H, H-18). ^{13}C NMR (400 MHz, *CDCl*$_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.3, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.6, C-8 35.4, C-9 40.5, C-10 34.7, C-11 20.6, C-12 40.2, C-13 40.9, C-14 56.2, C-15 31.8, C-16 81.6, C-17 61.2, C-18 16.6, C-19 23.3, C-20 38.2, C-21 14.4, C-22 118.4, C-23 44.1, C-23′ 61.5, C-24 33.6, C-24′ 14.7, C-25 73.9, CH_3COO-3 170.6, *C*H_3COO-3 21.5, CH_3COO-23′ 171.0, *C*H_3COO-23′ 21.0. MS (FAB) 457 [M^+-CH_3COO], 455, 397, 327, 281, 255, 207, 193, 147, 136, 91, 73(100%). HRMS (FAB) *observed* 457.3319 [M^+-CH_3COO] *required for* $C_{29}H_{45}O_4$ 457.3318.

(22*S*, 24*R*)-16β,22:22,25-diepoxy-23-methyliden-24-methyl-26,27-dinor-5β-choles-tan-3β-ol diacetate **(9)** yield 30.2 mg,



Scheme 3. Reaction mechanism for the bromination at C-23.

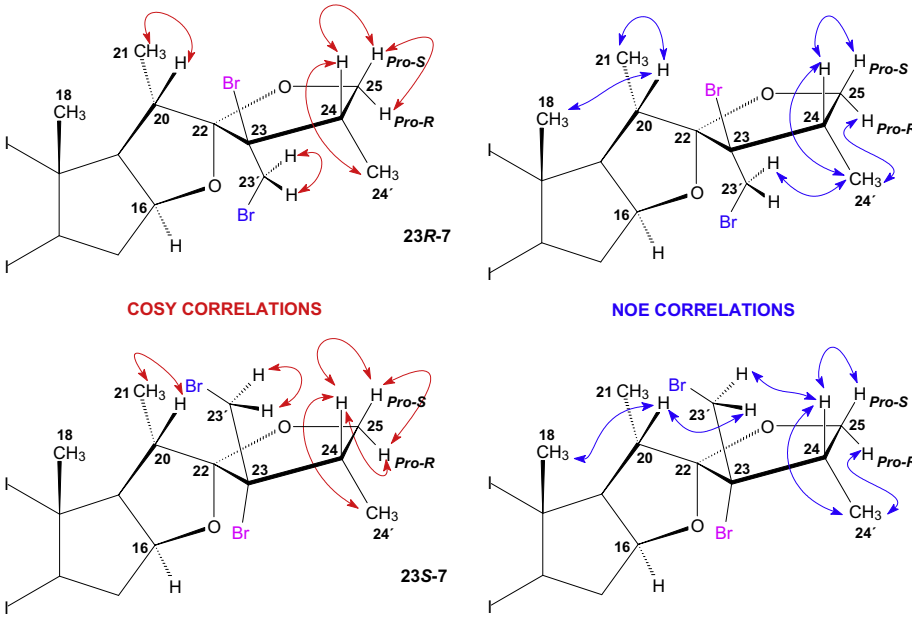
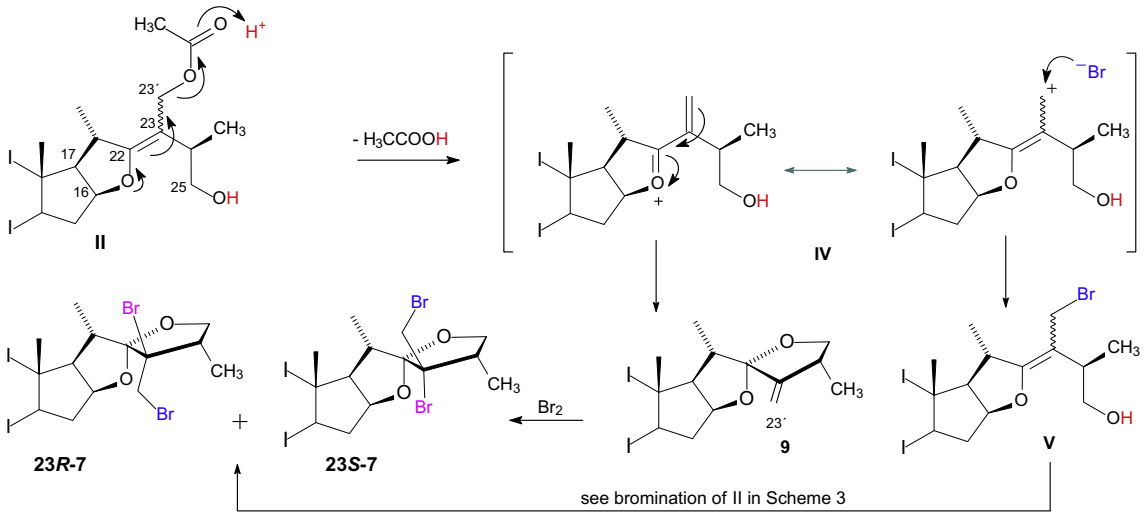
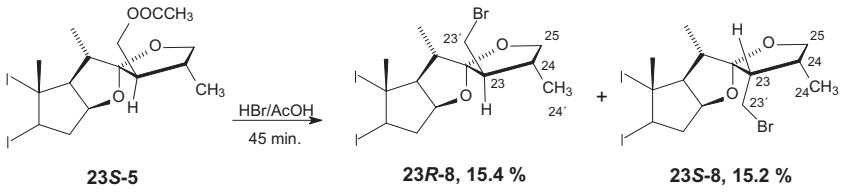


Fig. 5. Selected COSY and NOE correlations in the side chains of compounds **23*R*-7** (upper) and **23*S*-7** (lower).

(0.066 mmol, 6.6%). Yellow oil. ^{1}H NMR (400 MHz, *CDCl*$_3$, δ ppm): 5.26 (d, *J* = 3.0 Hz, 1H, H-23′a), 5.05 (d, *J* = 2.7 Hz, 1H, H-23′b), 4.71 (m, 1H, H-3), 4.49 (m, 1H, H-16), 4.03 (dd, *J* = 7.9, 7.9 Hz, 1H, H-25 *Pro-S*), 3.42 (dd, *J* = 10.3, 8.0 Hz, 1H, H-25 *Pro-R*), 2.67 (m, 1H, H-24), 2.30 (dq, *J* = 7.0, 7.0 Hz, 1H, H-20), 2.02 (s, 3H, CH_3-COO-3), 1.11 (d, *J* = 6.7 Hz, 3H, H-24′), 0.95 (s, 3H, H-19), 0.93 (d, *J* = 6.9 Hz, 3H, H-21), 0.87 (s, 3H, H-18). ^{13}C NMR (400 MHz, *CDCl*$_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.3, C-4 32.2, C-5 41.8, C-6 26.9, C-7 26.6, C-8 35.4, C-9 40.5, C-10 34.7, C-11 20.6, C-12 40.2, C-13 41.1, C-14 56.4, C-15 31.9, C-16 82.4, C-17 61.6, C-18 16.4, C-19 23.3, C-20 41.1, C-21 14.6, C-22 118.3, C-23 153.9, C-23′ 107.1, C-24 38.3, C-24′ 14.7, C-25 72.1, CH_3COO-3 170.6, *C*H_3COO-3



Scheme 4. Possible reaction mechanism for bromodeacetoxylation of **23*S*-5**.



Scheme 5. Reaction of **23*S*-5** with HBr in acetic acid.

21.4. MS (EI) 457 [MH^+], 456, 426, 396, 366, 329, 315, 284, 269, 255, 215, 161, 149, 137 (100%). HRMS (EI) *observed* 456.3250 [M^+] *required for* $C_{29}H_{44}O_4$ 456.3240.

6. Results and discussion

Treatment of the diacetate **23*S*-5** with $PyrBr_2$·HBr in acetic acid afforded the epimeric 23-brominated furospirostanes **23*R*-6** and **23*S*-6** together with the unexpected dibrominated compounds **23*R*-7** and **23*S*-7** (see Scheme 2).

Although the MS of compound **23*R*-6** does not show a molecular ion that shows the presence of a bromine, the occurrence of a fragment in *m*/*z* 515 may be interpreted as the product of the loss of Br from the molecular ion (M^+–Br or more likely MH^+–HBr). The signals of C-16, C-22 and C-26 in ^{13}C spectrum of compound **23*R*-6** evidence the integrity of the 16,22:22,25 diepoxy moiety, while the

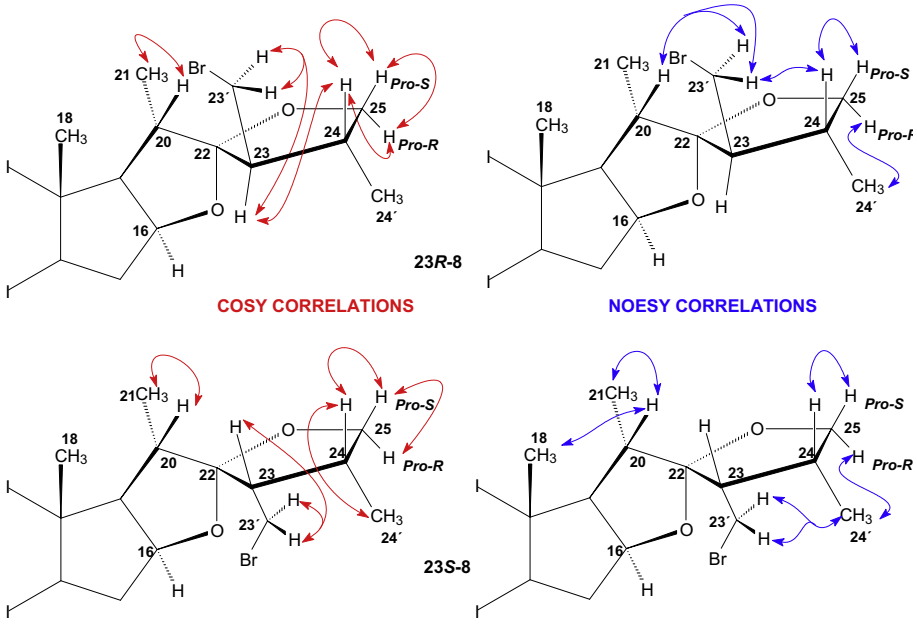
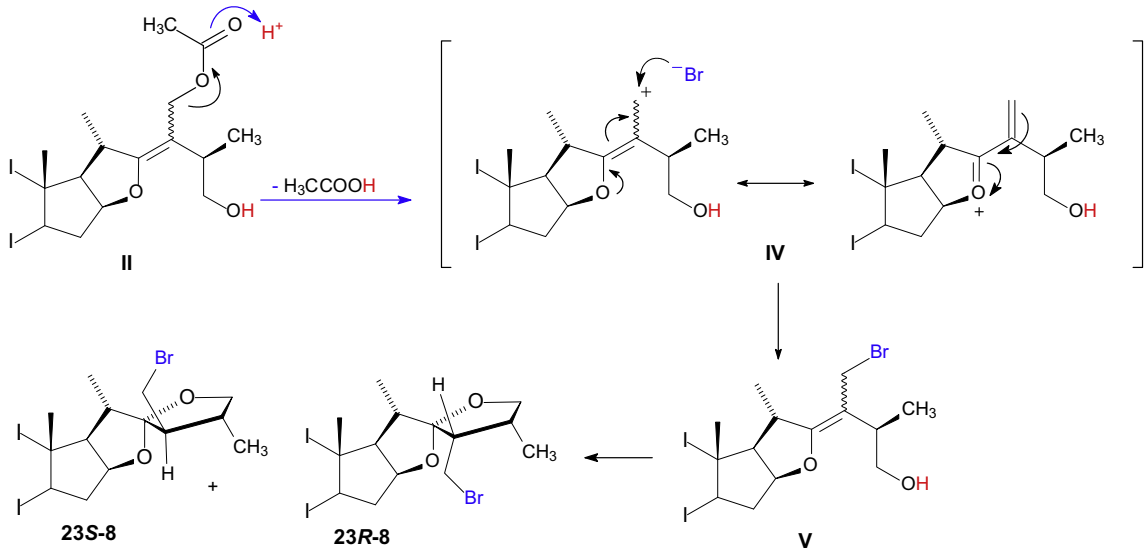
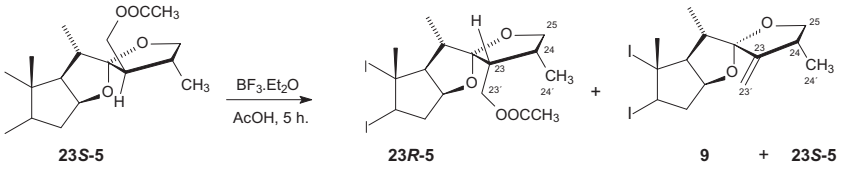


Fig. 6. Selected COSY and NOESY correlations observed in compounds **23*R*-8** and **23*S*-8**.



Scheme 6. Possible reaction mechanism of the substitution of the C-23′-acetoxy moiety.



Scheme 7. BF3·Et^{2}O-catalyzed reaction of **23*S*-5** in acetic acid.

downfield shift of the signal of C-23 suggests the substitution of H-23 by a more electronegative atom that, according the reaction conditions, must be no other than a bromine atom. In addition, the substitution of H-23 is evidenced by the disappearance of its NMR signal and the conversion of the H-23′ signals from an AB-X system into a doublet, product of the H-23′a ↔ H-23′b geminal coupling, (J = 11.8 Hz). The observed COSY and NOE correlations also corroborate the proposed structure (see Fig. 3). In particular, the NOE correlations of each H-23′ with H-20 and H-24 in the respective β-side of rings E and F prove their spatial proximity only possible if the acetoxymethyl moiety that bears the H-23′ diaterotopic pair is placed in the β-side that results on the *R* configuration at C-23. Similar conclusions can be drawn from the MS and NMR spectra of compound **23*S*-6**; the observed NOE correlation between the H-24′ methyl group in the α-side of F-ring and the H-23′ diasterotopic pair, indicates the α-orientation of the exocyclic acetoxymethyl moiety and consequently the *S*-configuration at C-23 (see Fig. 3).

X-ray studies on monocrystals of both diasteromers **23*R*-6** and **23*S*-6** corroborated the proposed structures and configuration at C-23 (see Fig. 4).

The occurrence of the diastereomeric compounds **23*R*-6** and **23*S*-6** can be justified by the acid catalyzed F-ring opening in compound **23*S*-5** that leads to the intermediate **I**. Bromination at C-23 leads to the mixture of the diastereomeric intermediates **23*R*-III** and **23*S*-III** that on F ring closure produce the observed mixture of **23*R*-6** and **23*S*-6** (see Scheme 3).

As described for **23*R*-6** and **23*S*-6**, in the case of the dibrominated compounds **23*R*-7** and **23*S*-7**, the absence of the signal corresponding to H-23 and the doublet multiplicity of the diasterotopic protons at C-23′ suggest the presence of the bromine atom at C-23. The occurrence of monobrominated fragments in *m*/*z* 535 and 537 may be interpreted as the product of the loss of Br from the molecular ions (M^+−Br or more likely MH^+−HBr) of the dibrominated compounds. Additionally the change on the ^{1}H and ^{13}C signals associated to the exocyclic methylene C-23′ (*compared to those of the starting material **23R-5** and the monobrominated compounds **23R-6** and **23S-6***) and well as the disappearance of the ^{1}H and ^{13}C signals associated to the acetoxy moiety formerly attached to C-23, evidence the presence of a different atom at C-23 that, according to the reaction conditions, must be no other than a bromine.

NOESY and COSY correlations observed in compounds **23*R*-7** and **23*S*-7** corroborated the integrity of the side chain as well as the configuration at C-23. In particular, for compound **23*R*-7** the observed between H-23′ ↔ H-24′ NOESY correlation indicates the α-orientation of the C-23′ exocyclic methylene and, as a consequence, the *R*-configuration at C-23. On the other hand, the observed H-23′ ↔ H-20 and H-23′ ↔ H-24 NOESY correlations indicate the β-orientation of the C-23′ exocyclic methylene in compound **23*S*-7** and consequently its *S*-configuration at C-23 (see Fig. 5).

The unexpected occurrence of the dibrominated compounds **23*S*-7** and **23*R*-7** can be rationalized as follows. Elimination of the allylic acetate in the intermediate **II** may lead to the intermediate **IV** that undergoes F ring closure to produce the alkene **9** that on bromination leads to the observed dibrominated compounds. In addition, attack of the bromide anion to an allylic carbocation at C-23́ may lead to the intermediate **V** that undergoes bromination at C-23 and F-ring closure as described above (see Scheme 4).

To gain a more detailed view on the proposed mechanisms, the possibility of the acid substitution of the acetoxy moiety at

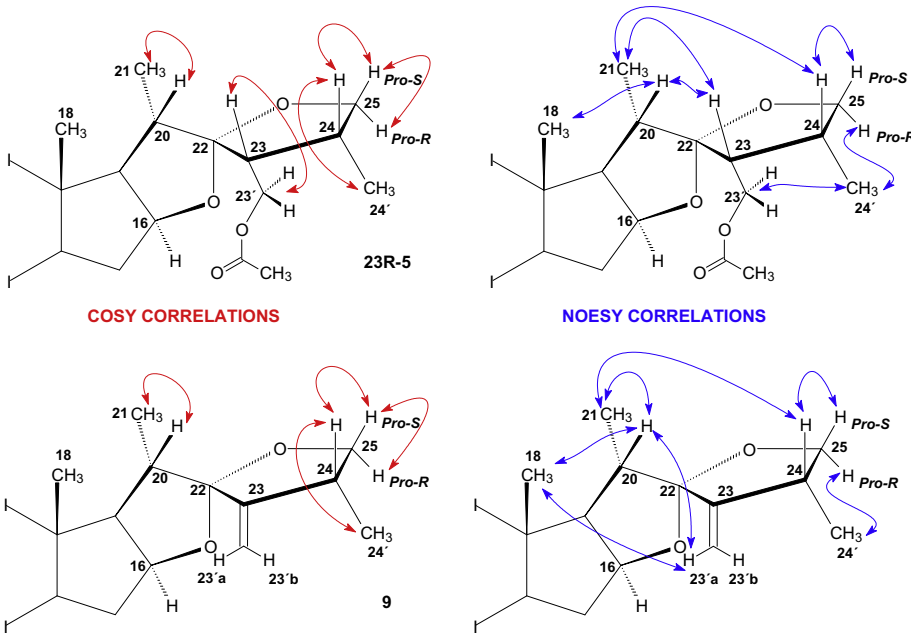
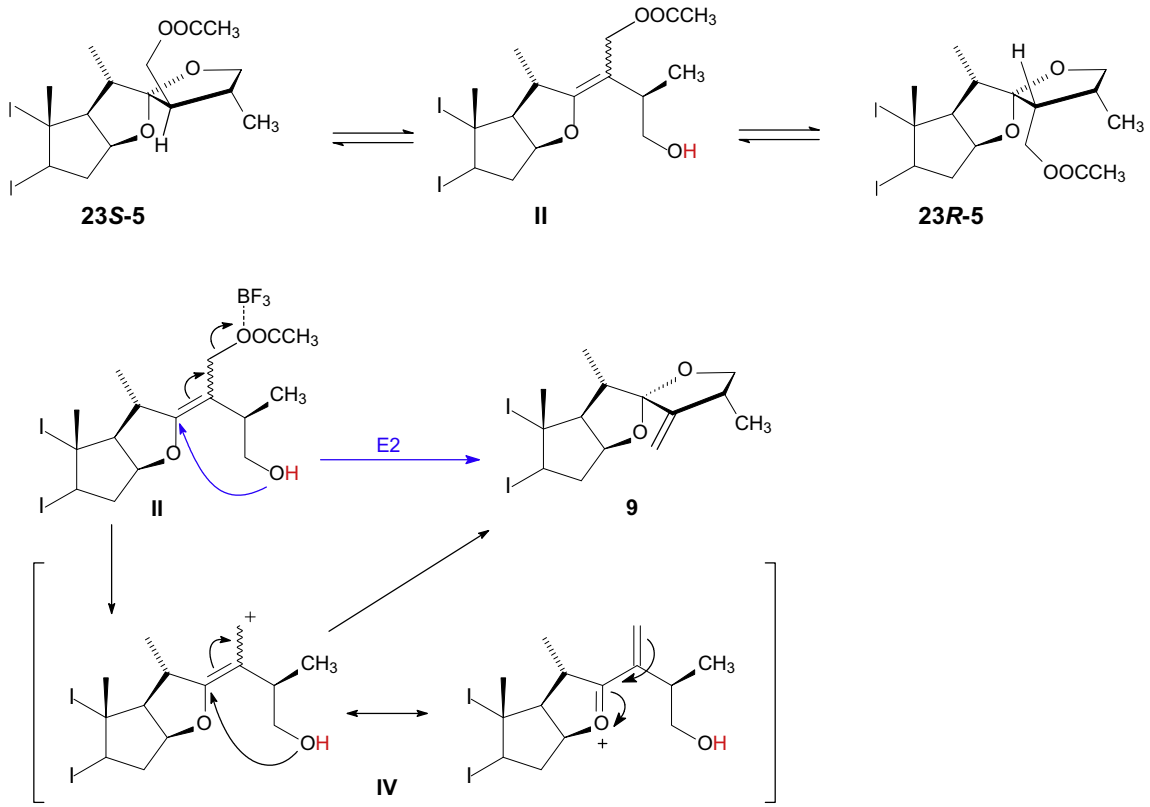


Fig. 7. Selected COSY and NOESY correlations in compounds **23S-6** and **9**.



Scheme 8. Possible mechanism for C-23 epimerization and elimination reactions.

C-23′was explored. Treatment of **23*S*-5** with HBr in acetic acid produced complex mixture from which the C-23′ brominated compounds **23*S*-8** and **23*R*-8** were isolated in low yields (see Scheme 5).

The Mass spectra of both 23′-brominated compounds are characterized by the presence of molecular ions (MH^+, *m*/*z* 539 and 537) that indicate the incorporation of a bromine atom. Disappearance of the ^1H and ^{13}C signals corresponding to the acetoxy moiety formerly attached to C-23′ indicates the presence of a bromine atom at this position. As in the preceding compounds, the integrity of the spiroketal moiety and the configuration at C-23 in compounds **23*R*-8** and **23*S*-8** can be verified by the presence of the ^1H and ^{13}C signals as well as the observation of the COSY and NOESY correlations (see Fig. 6).

The occurrence of the C-23′ brominated compounds **23*R*-8** and **23*S*-8** evidences the substitution of the acetoxy group attached to C-23′. Acid catalyzed dissociation of **II** leads to the allylic cation **IV** that on reaction with the bromine anion produces **V**. F-ring closure in **V** leads to the observed substitution products **23*R*-8** and **23*S*-8**. The observed substitution process strongly suggests the existence of the intermediates **II**, **IV** and **V** in this and the above described reactions (see Scheme 6).

Finally, when **23*S*-5** was treated with $BF_3{\cdot}Et_2O$ in acetic acid to explore the acid-catalyzed inversion at C-23, a mixture of the starting material **23*S*-5**, its C-23 epimer **23*R*-5** and the alkene **9** was obtained (see Scheme 7).

Again the integrity of the side chains of compounds **23*R*-5** and **9**, can be verified by observation of the ^1H and ^{13}C signals corresponding to this fragment as well as the present COSY and NOESY correlations. The configuration at C-23 in compound **23*R*-5** can be verified by observation of the H-23′ ↔ H-24′ both in the α-side of the F-ring (see Fig. 7). In particular the alkene **9** can be recognized by the absence of the signals corresponding to the acetoxy moiety formerly present at C-23′ as well as the new ^1H and ^{13}C signals corresponding to the new exocyclic double bond.

Protonation of the intermediate **II** from the *Re* or *Si* sides of C-23, followed by F-ring closure explain the production of the **23*R*-5**/**23*S*-5** mixture. On the other hand, $BF_3{\cdot}Et_2O$-catalyzed concerted elimination/F ring closure may justify the occurrence of the alkene **9**. Alternatively the olefin **9** can be produced through F ring closure in the allylic carbocation **IV** (see Scheme 8).

7. Conclusions

We have found that the presence of the C-23′ acetoxy moiety confers a special reactivity to the furospirostane side chain. The F-ring opening produces a Δ^{22}-intermediate in which the C-23′-acetoxy is placed in position allowing its substitution or elimination. This reactivity feature opens new and useful possibilities for the transformation of the 16β,22:22,25-diepoxy-23-acetoxy-methyl-24-methyl side chain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2013.05.014.

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