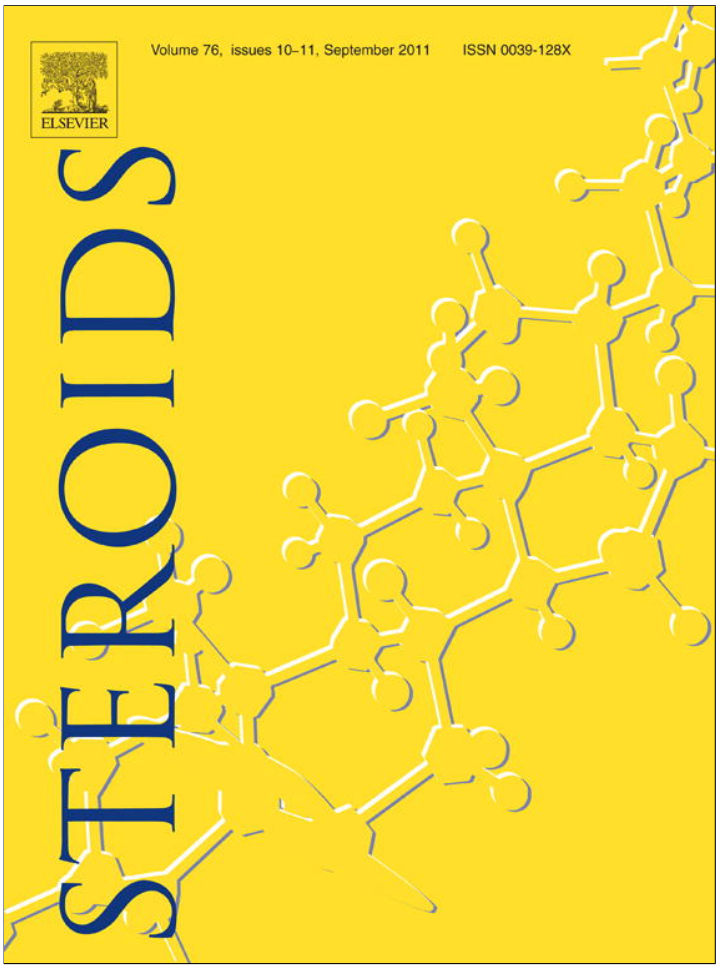

Steroids 76 (2011) 1021–1031

Contents lists available at ScienceDirect

Steroids

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On the reactivity of 23-methoxycarbonyl furospirostanes

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ARTICLE INFO

Article history:
Received 15 March 2011
Received in revised form 31 March 2011
Accepted 1 April 2011
Available online 14 April 2011

Keywords:
Furospirostanic side chain
Brønsted acid-catalysed reactions
Lewis acid-catalysed reactions
Bromination
Deuteration
Rearrangements

ABSTRACT

Brønsted and Lewis acid-catalysed reactions of the 23-methoxycarbonyl furospirostanic side chain are described. While bromination, deuteration and $BF_3{\cdot}Et_2O$/AcOH treatment involve regioselective F-ring opening with exclusive participation of Δ^{22}-furostenic intermediates, $BF_3{\cdot}Et_2O/Ac_2O$ treatment leads to irreversible E- or F-ring cleavage.

1. Introduction

Spiroketals constitute a large family of naturally occurring compounds that have been isolated from both terrestrial and marine organisms. In general the spiroketal moiety is a prevalent structure in a large number of important natural products that have attracted much interest, not only for their wide spectrum of biological activity, but also for their interesting reactivity and usefulness in the preparation of other bioactive compounds [1–3].

Steroids bearing spiroketal side chains are widespread in both the natural and synthetic domains. Spirostanic sapogenins (see Fig. 1) the reactivity of which has produced a wide variety of interesting reactions [4–24] are characterized by the presence of a 16β,22:22,26-diepoxy moiety in the side chain, being 1,6-dioxaspiro[4.5] decane derivatives. Such compounds have been subject of much research due to their intrinsic biological activity [25–30] as well as their usefulness as starting materials for the synthesis of bioactive compounds such as sex and adrenocortical hormones, [4] ecdysteroids, [31] plant growth stimulators, [32–38] and cytotoxic steroids, [39–45] among many others.

It is well known that, in acid media, spirostanic sapogenins present an equilibrium in which the spiroketalic side chain is opened to the oxacarbenium ion **I** that may lose a proton from either C-20 or C-23 to produce Δ^{20} or Δ^{22} enol ethers (see Scheme 1) [4]. Most of the reactivity of the spirostanic side chain can be justified on the basis of these two enolic forms and the oxacarbenium ion **I**.

Furospirostanes, a smaller and somewhat less known family of steroids bearing a 16β,22:22,25-diepoxy moiety in the chain, may be considered 1,6-dioxaspiro[4.4]nonane derivatives, and includes compounds with antitumor activity as the ritterazines **(4),** cephalostatines **(5)** [41–45], or hippuristanols **(6–8)** [46–49], among others (see Fig. 2).

Fuchs and coworkers correlated the cytotoxicity of cephalostatins and their synthetic analogues with that of the potent anti-tumor steroid OSW-1 and hypothesized that the cytotoxic activity of such compounds may be connected with the possibility of the generation of oxacarbenium ions around C-22 [50–52]. In the light of this hypothesis, the fact that other furospirostanes with different 16β,22:22,25-diepoxy side chains (i.e. hippuristanols **6–8**, Fig. 2) and even steroid sapogenins (**1–3**, Fig. 1) with the 16β,22:22,26-diepoxy side chain, (*all theoretically able to produce oxacarbeniums ions around C-22*), have shown toxic activity against different cancer cell lines, should not be considered a mere coincidence. On the contrary, this fact supports Fuchsĭs hypothesis, and constitute an invitation to the study of the biological activity and reactivity of other compounds bearing slightly or even drastically modified side chains that still are able to generate oxacarbeniums ions around C-22.

As a part of our project to explore the reactivity of steroid bearing spiroketals side chains, we have found that treatment of 23-oxo-spirostanes with diacetoxyiodobenzene (DIB) and KOH in methanol produces a *quasi*-Favorskii F-ring contraction that leads to 23-methoxycarbonyl-furospirostanes (see Scheme 2) [10].

This has led us to initiate a program to study the reactivity of the derived compounds as well as their usefulness as starting materials for the synthesis of other potentially bioactive steroids bearing

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doi:10.1016/j.steroids.2011.04.006

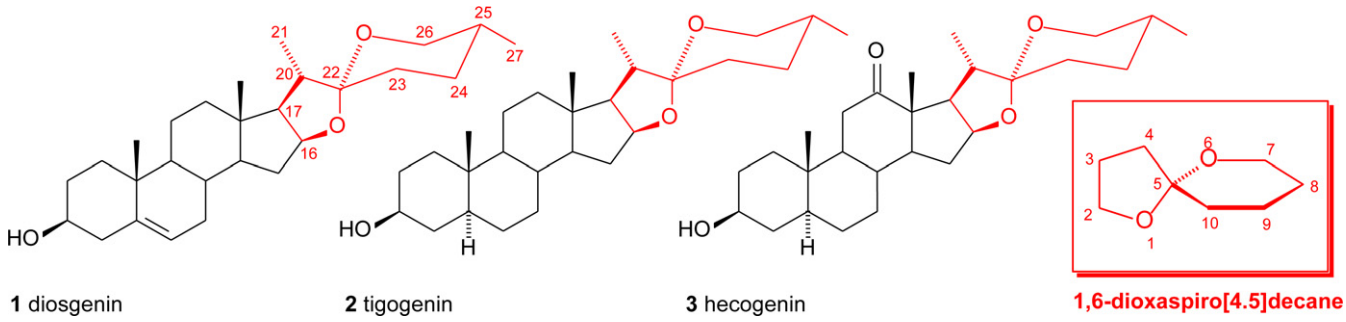
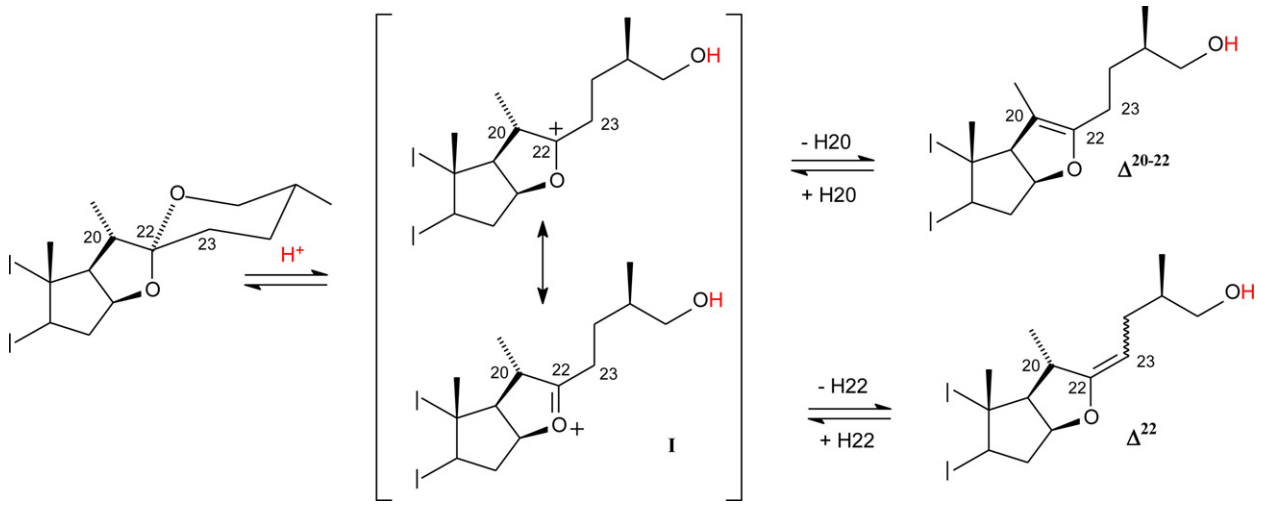


Fig. 1. Some naturally occurring spirostanic sapogenins.



Scheme 1.

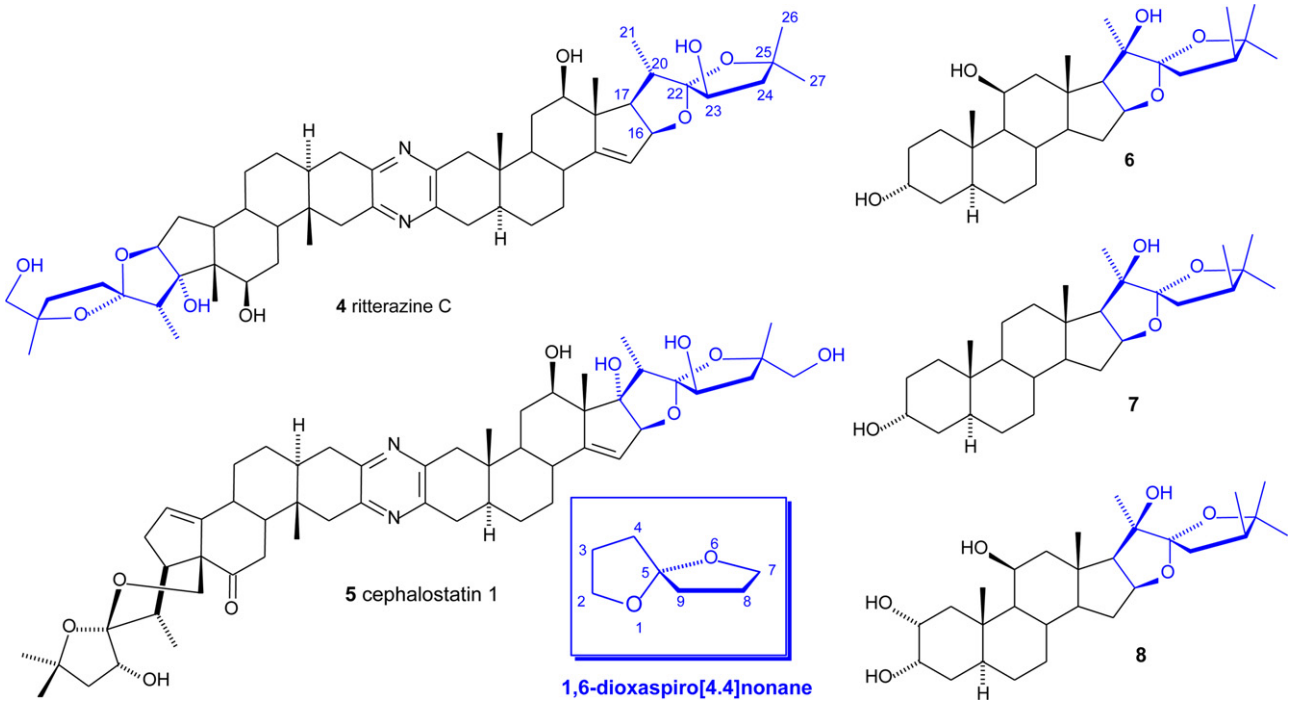
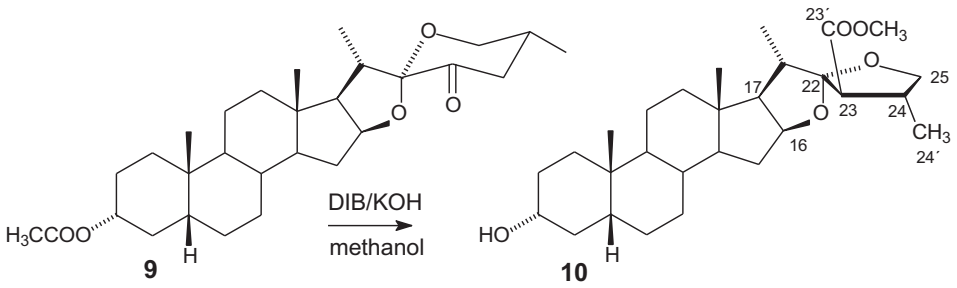


Fig. 2. Some furospirostanes with cytotoxic activity against cancer cells.



Scheme 2.

modified spiroketalic side chains. Herein we report on the reactivity of the 23-methoxycarbonyl-16β,22:22,25-diepoxy side chain under both Lewis and Brønsted acid catalysis.

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. Melting points were measured on a Melt-Temp II equipment and are uncorrected. 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 7.26 ppm for ^1H 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 (^1H–^1H) and heteronuclear (^1H–^{13}C) correlation techniques, which included ^1H–^1H COSY, ^1H–^1H Nuclear Overhauser Effect Spectroscopy (NOESY), and Heteronuclear Single Quantum Correlation (HSQC). All 2D NMR spectra were recorded using the standard pulse sequences and parameters recommended by the manufacturer.

(*22S,23R,24R*)-16β,22:22,25-diepoxy-23-methoxycarbonyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate (11). Acetic anhydride 0.8 mL was added to a solution of **10** [10] in pyridine (8 mL), the mixture was stirred overnight, poured into a mixture of ice and 50 mL of 10% HCl. The mixture was extracted with ethyl acetate (2 × 40 mL) and the organic layer was washed with 10% HCl solution (2 × 25 mL), 5% $CuSO_4$ solution (3 × 25 mL), water (2 × 30 mL), dried (anh. Na_2SO_4) and evaporated to afford the acetylated product **11** (1.06 g, 2.11 mmol, 74.8%). m.p. 172–174 °C (acetone/hexane). ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 4.69 (m, 1H, H-3), 4.40 (ddd, J = 7.7, 7.7, 6.0 Hz, 1H, H-16), 4.01 (dd, J = 7.7, 7.7 Hz, 1H, H-25 *Pro-S*), 3.71 (s, 3H, CH_3O), 3.46 (dd, J = 10.1, 8.1 Hz, 1H, H-25 *Pro-R*), 2.82 (d, J = 9.3 Hz, 1H, H-23), 2.64 (m, 1H, H-24), 2.10 (dq, J = 6.8, 6.8 Hz, 1H, H-20), 2.00 (s, 3H, CH_3COO-3), 1.96 (m, 1H, H-15α), 1.01 (d, J = 6.6 Hz, 3H, H-24′), 0.92 (s, 3H, H-19), 0.92 (d, J = 6.9 Hz, 3H, H-21), 0.74 (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 35.5, C-9 40.4, C-10 34.7, C-11 20.5, C-12 39.9, C-13 41.1, C-14 56.2, C-15 31.8, C-16 81.4, C-17 62.7, C-18 16.0, C-19 23.3, C-20 37.4, C-21 15.5, C-22 119.3, C-23 59.6, C-23′ 172.4, C-24 37.5, C-25 72.5, C-24′ 15.4, $CH_3\underline{C}OO$-3 170.5, $\underline{C}H_3COO$-3 21.4, $\underline{C}H_3O$-23′ 51.8. **MS (FAB)**: 503 MH^+, 471, 469, 425, 409, 315, 255 (100%). Anal: $C_{27}H_{44}O_4$ requires C, 71.68%; H, 9.22%/. Found: C, 71.61%; H, 9.31%.

2.1. Reactions under $BF_3{\cdot}Et_2O$ catalysis

Reaction with $BF_3{\cdot}Et_2O$ in acetic acid: $BF_3{\cdot}Et_2O$ (1 mL, 3.8 mmol) was added to a solution of **11** (251.4 mg, 0.5 mmol) in acetic acid (5 mL) and the mixture was stirred for ten days at room temperature. Ice-water (50 mL) was added and the mixture was extracted with ethyl acetate (2 × 25 mL). The organic layer was washed with water (5 × 50 mL), dried (anh. Na_2SO_4) and evaporated. The produced syrup was purified using a pressurized chromatographic column packed with silicagel (7.5 g) and employing 15/1 hexane/ethyl acetate mixture for elution to afford the syrupy unsaturated lactone ***E*-12** (206.3 mg, 0.44 mmol, 88%). (*22E,24R*)-3α-acetoxy-24-methyl-26,27-dinor-5β-furost-22-en-23,25-carbolactone (***E*-12**). **^1H NMR** (300 MHz, $CDCl_3$, δ ppm): 5.04 (ddd, J = 7.4, 7.4, 4.1 Hz, 1H, H-16), 4.71 (m, 1H, H-3), 4.33 (dd, J = 8.6, 8.6 Hz, 1H, H-25 *Pro-S*), 3.81 (dd, J = 8.8, 4.2 Hz, 1H, H-25 *Pro-R*), 3.65 (m, 1H, H-20), 3.14 (m, 1H, H-24), 2.23 (m, 1H, H-15α), 2.02 (s, 3H, $\underline{C}H_3COO$), 1.24 (d, J = 7.2 Hz, 3H, H-21), 1.18 (d, J = 6.8 Hz, 3H, H-24′), 0.92 (s, 3H, H-19), 0.57 (s, 3H, H-18). **^{13}C NMR** (75 MHz, $CDCl_3$, δ ppm): C-1 35.0, C-2 26.6, C-3 74.2, C-4 32.2, C-5 41.7, C-6 26.8, C-7 26.5, C-8 35.2, C-9 40.6, C-10 34.7, C-11 20.2, C-12 38.3, C-13 41.7, C-14 55.4, C-15 33.5, C-16 88.1, C-17 62.1, C-18 13.3, C-19 23.2, C-20 36.8, C-21 19.2, C-22 177.4, C-23 97.4, C-23′ 172.5, C-24 32.5, C-25 72.5, C-24′ 20.1, $CH_3\underline{C}OO$-3 170.5, $\underline{C}H_3COO$-3 21.4. **MS (EI 70 eV)**: 471 MH^+, 410, 255, 193 (100%), HR**MS (EI)** observed 470.3014 M^+; required for $C_{29}H_{42}O_5$ 470.3032.

Reaction with $BF_3{\cdot}Et_2O$ and acetic anhydride: $BF_3{\cdot}Et_2O$ (2 mL, 7.6 mmol) was added to a suspension of **11** (460 mg, 0.92 mmol) in acetic anhydride (15 mL), the mixture was stirred at room temperature for 45 min and poured into ice-water (60 mL). The resulting mixture was extracted with ethyl acetate (2 × 50 mL), the organic layer was washed with water (2 × 50 mL), dried (anh. Na_2SO_4) and evaporated to afford a syrupy residue that was purified in pressurized chromatographic column packed with silicagel (15 g) employing 20/1 hexane/ethyl acetate mixture for elution to afford 118 mg (0.22 mmol, 23.9%) of the diacetylated cyclic enol ether **13** and 99.8 mg (0.18 mmol, 19.6%) of diacetylated furostene ***E*-14**

(*24R*)-22,25-epoxy-24-methyl-26,27-dinor-5β-cholest-22-en-3α,16β-diol diacetate **(13)**. **^1H NMR** (300 MHz, $CDCl_3$, δ ppm): 5.04 (m, 1H, H-16), 4.71 (m, 1H, H-3), 4.36 (t, J = 9.2 Hz, 1H, H-25 *Pro-S*), 3.99 (dd, J = 8.9, 4.8 Hz, 1H, H-25 *Pro-R*), 3.90 (dq, J = 12.3, 7.1 Hz, 1H, H-20), 3.69 (s, 3H, CH_3O), 3.12 (m, 1H, H-24), 2.33 (m, 1H, H-15α), 2.03 (s, 3H, $\underline{C}H_3COO$-3), 1.85 (s, 3H, $\underline{C}H_3COO$-16), 1.18 (d, J = 6.9 Hz, 3H, H-21), 1.09 (d, J = 6.7 Hz, 3H, H-24′), 0.93 (s, 3H, H-19), 0.89 (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 42.7, C-6 26.8, C-7 26.1, C-8 35.3, C-9 40.4, C-10 34.6, C-11 20.6, C-12 40.1, C-13 41.7, C-14 54.1, C-15 34.9, C-16 75.2, C-17 55.6, C-18 12.9, C-19 23.2, C-20 29.9, C-21 18.3, C-22 176.2, C-23 104.9, C-23′166.3, C-24 36.5, C-24′ 19.8, C-25 77.9, CH_3O 50.5, $CH_3\underline{C}OO$-3 170.6, $CH_3\underline{C}OO$-25 170.7, $\underline{C}H_3COO$-3 21.4, $\underline{C}H_3COO$-16 20.9. **MS (70 eV):** 544 MH^+, 484, 470, 424, 409, 377, 315, 256, 255 (100%). **HRMS (EI)** *observed* 544.3400 MH^+; *required for* $C_{27}H_{44}O_4$ 544.3400.

(*22Z,24R*)-24-methyl-26,27-dinor-5β-furost-22-en-3α,25-diol diacetate **(*E*-14)**. **^1H NMR** (300 MHz, $CDCl_3$, δ ppm): 4.95 (ddd, J = 7.5, 7.5, 4.0 Hz, 1H, H-16), 4.67 (m, 1H, H-3), 4.11 (d, J = 7.7 Hz, 2H, H-25), 3.65 (s, 3H, CH_3O), 3.59 (m, 1H, H-20), 3.21 (tq, J = 7.4,

7.4 Hz, 1H, H-24), 2.19 (td, *J* = 14.0, 7.2 Hz, 1H, H-15α), 1.99 (s, 3H, $\underline{C}H_3COO$-3), 1.96 (s, 3H, $\underline{C}H_3COO$-25), 1.15 (d, *J* = 7.1 Hz, 3H, H-21), 1.08 (d, *J* = 7.1 Hz, 3H, H-24′), 0.89 (s, 3H, H-19), 0.56 (s, 3H, H-18). **^{13}C NMR** (75 MHz, MHz, $CDCl_3$, δ ppm): C-1 34.9, C-2 26.7, C-3 74.3, C-4 32.1, C-5 41.7, C-6 26.7, C-7 26.5, C-8 35.1, C-9 40.5, C-10 34.6, C-11 20.1, C-12 38.3, C-13 41.7, C-14 55.3, C-15 33.4, C-16 86.7, C-17 62.1, C-18 13.2, C-19 23.2, C-20 38.5, C-21 20.1, C-22 179.1, C-23 100.8, C-23′ 168.9, C-24 31.1, C-25 67.7, C-24′ 15.3, CH_3O 50.7, $CH_3\underline{C}OO$-3 170.8, $CH_3\underline{C}OO$-25 171.5, $\underline{C}H_3COO$-3 21.3, $\underline{C}H_3COO$-25 20.9. **MS (EI 70 eV)**: 544 MH^+, 484, 471, 452, 410, 315, 255, 207, 193 (100%). **HRMS (EI)** *observed* 544.3388 MH^+; *required for* $C_{27}H_{44}O_4$ 544.3400.

2.2. Reaction under Brønsted acid catalysis

Bromination with PyHBr·Br$_2$ in acetic acid: $PyHBr{\cdot}Br_2$ (255.7 mg, 0.8 mmol) was added to a warm (50 °C) solution of **11** (126 mg, 0.25 mmol) in acetic acid (5 mL), and the resulting mixture was stirred at 50 °C for 30 min. Cold water (15 mL) was added and the produced solid was filtered off and washed with plenty of water to afford 141 mg, (0.24 mmol, 96%) of a 1/2.4 mixture of the diasteromeric brominated products **23*R*-15** and **23*S*-15**, (relation determined by relative integration of the H-26 *pro-S* signals of both epimers, see supplementary information). Analytical samples of both compounds were obtained in a pressurized chromatographic column packed with silicagel (20 g) and employing 19/1 hexane/ethyl acetate mixture for elution.

(*22S,23R,24S*)-16β,22:22,25-diepoxy-23-bromo-23-methoxycarbonyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate **(23*R*-15)**. **m.p.** 195–196 °C, *from hexane/ethyl acetate*. **^{1}H NMR** (400 MHz, $CDCl_3$ δ ppm): 4.70 (m, 1H, H-3), 4.48 (m, 1H, H-16), 4.14 (dd, *J* = 7.6, 7.6 Hz, 1H, H-25 *Pro-S*), 3.77 (dd, *J* = 8.2, 6.5 Hz, 1H, H-25 *Pro-R*), 3.74 (s, 3H, CH_3O), 2.92 (dq, *J* = 6.7, 6.7 Hz, 1H, H-20), 2.85 (m, 1H, H-24), 2.01 (s, 3H, $\underline{C}H_3COO$), 1.30 (d, *J* = 7.1 Hz, 3H, H-24′), 1.17 (d, *J* = 6.9 Hz, 3H, H-21), 0.94 (s, 3H, H-19), 0.85 (s, 3H, H-18). **^{13}C NMR** (100 MHz, $CDCl_3$ δ ppm): C-1 35.2, C-2 26.7, C-3 74.3, C-4 32.4, C-5 42.0, C-6 27.0, C-7 26.7, C-8 35.5, C-9 40.7, C-10 34.8, C-11 20.7, C-12 40.4, C-13 41.4, C-14 56.2, C-15 32.0, C-16 82.4, C-17 63.2, C-18 16.2, C-19 23.3, C-20 40.1, C-21 17.0, C-22 119.0, C-23 69.3, C-23′ 167.3, C-24 48.0, C-24′ 15.2, C-25 72.4, CH_3O 52.3, $CH_3\underline{C}OO$ 170.4, $\underline{C}H_3COO$ 21.3. **MS (FAB)** 583 MH^+, 581 MH^+, 579, 534, 518, 503, 458, 401, 385, 355, 341, 327 (100%). Anal: $C_{30}H_{45}BrO_6$ requires C, 61.96; H, 7.80/. Found: C, 61.48; H, 7.63.

A crystal of compound **23*R*-15** suitable for X-ray diffraction studies was obtained from a hexane/ethyl acetate solution. Compound **23*R*-15** crystallized as a monoclinic system with the space group P 2(1); *a* = 12.9040(4) Å, *b* = 7.8060(2) Å, *c* = 14.4940(3) Å, β = 96.824(2)° and *V* = 1449.62(7) Å3. X-ray diffraction measurements were performed on an Oxford Diffraction Atlas (Gemini) diffractometer with Mo K(radiation (= 0.71073 Å. Data collection routine and data reduction were carried out with CrysAlisPro [53]. The structures compound **23*R*-15** was solved using SIR2004 [54], and refined using SHELXL-97 [55]. All non-hydrogen atoms were anisotropically refined and the hydrogen atoms were found in difference Fourier maps, placed at geometrically calculated positions and refined using the riding model. The obtained bond lengths and angles are normal and are available from the electronic supporting information (CIF file) for the structure. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center as supplementary material numbers CCDC 792938. Copy of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. E-mail: deposit@ccdc.cam.ac.uk.

(*22S,23S,24S*)-16β,22:22,25-diepoxy-23-bromo-23-methoxycarbonyl-24-methyl-26,27-dinor-5β-cholestan-3α-ol acetate **(23*S*-15)**. ^{1}H NMR (400 MHz, $CDCl_3$ δ ppm): 4.69 (m, 1H, H-3), 4.49 (m, 1H, H-16), 4.03 (dd, *J* = 7.8, 7.8 Hz, 1H, H-25 *Pro-S*), 3.77 (s, 3H, OCH_3), 3.67 (dd, *J* = 8.7, 8.1 Hz, 1H, H-25 *Pro-R*), 2.91 (m, 1H, H-24), 2.20 (dq, *J* = 6.7, 6.7 Hz, 1H, H-20), 2.00 (s, 3H, $\underline{C}H_3COO$), 1.13 (d, *J* = 6.6 Hz, 3H, H-24′), 0.94 (d, *J* = 6.8 Hz, 3H, H-21), 0.92 (s, 3H, H-19), 0.79 (s, 3H, H-18). **^{13}C NMR** (100 MHz, $CDCl_3$, δ ppm): C-1 35.1, C-2 26.6, C-3 74.2, C-4 32.3, C-5 41.9, C-6 27.0, C-7 26.7, C-8 35.5, C-9 40.6, C-10 34.8, C-11 20.6, C-12 40.7, C-13 41.6, C-14 56.2, C-15 31.8, C-16 81.6, C-17 63.5, C-18 15.8, C-19 23.3, C-20 37.6, C-21 16.2, C-22 117.8, C-23 71.7, C-23′ 169.3, C-24 40.2, C-25 71.6, C-24′ 16.5, CH_3O 53.1, $CH_3\underline{C}OO$ 170.4, $\underline{C}H_3COO$ 21.3. **MS (EI)** 471 [MH^+–($CH_3O^\bullet$)–($Br^\bullet$)], 443, 344, 329, 284, 269, 255, 183, 152107, 83, 69, **HRMS (CI)** *observed* 581.2457 MH^+ *required for* $C_{30}H_{46}Br^{(79)}O_6$ 581.2478.

Deuteration experiment: ^{1}H NMR of a 0.1 M solution of **11** (50 mg in 1 mL of D_3CCOOD) was registered shortly after preparation of the solution, and after 24 h, (see Fig. 9, spectrum **A**). Then DCl (10 μL, 99% D, 35% en D_2O) was added and the 1H spectra were registered every 5 min during 3 h (see Fig. 9, spectra **B** to **E**).

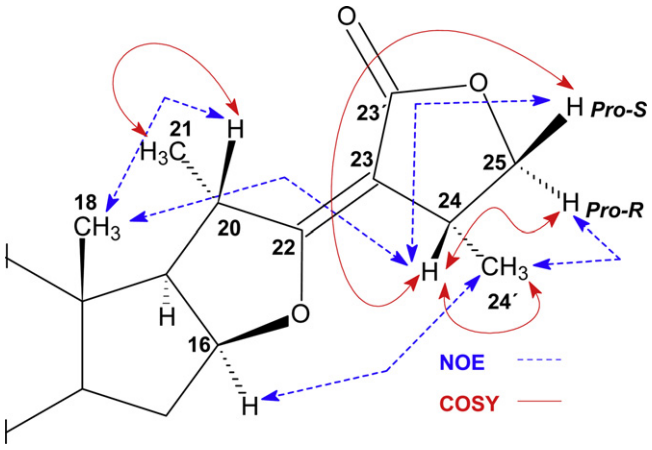


Fig. 3. Selected NOE and COSY correlations in the side chain of compound ***E*-12**.

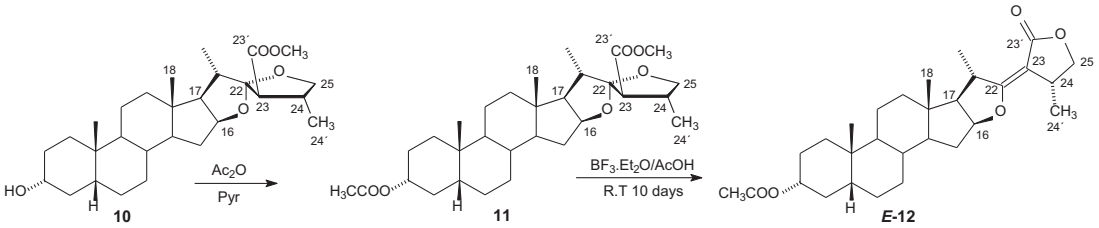
3. Results and discussion

3.1. Reactions under $BF_3{\cdot}Et_2O$ catalysis

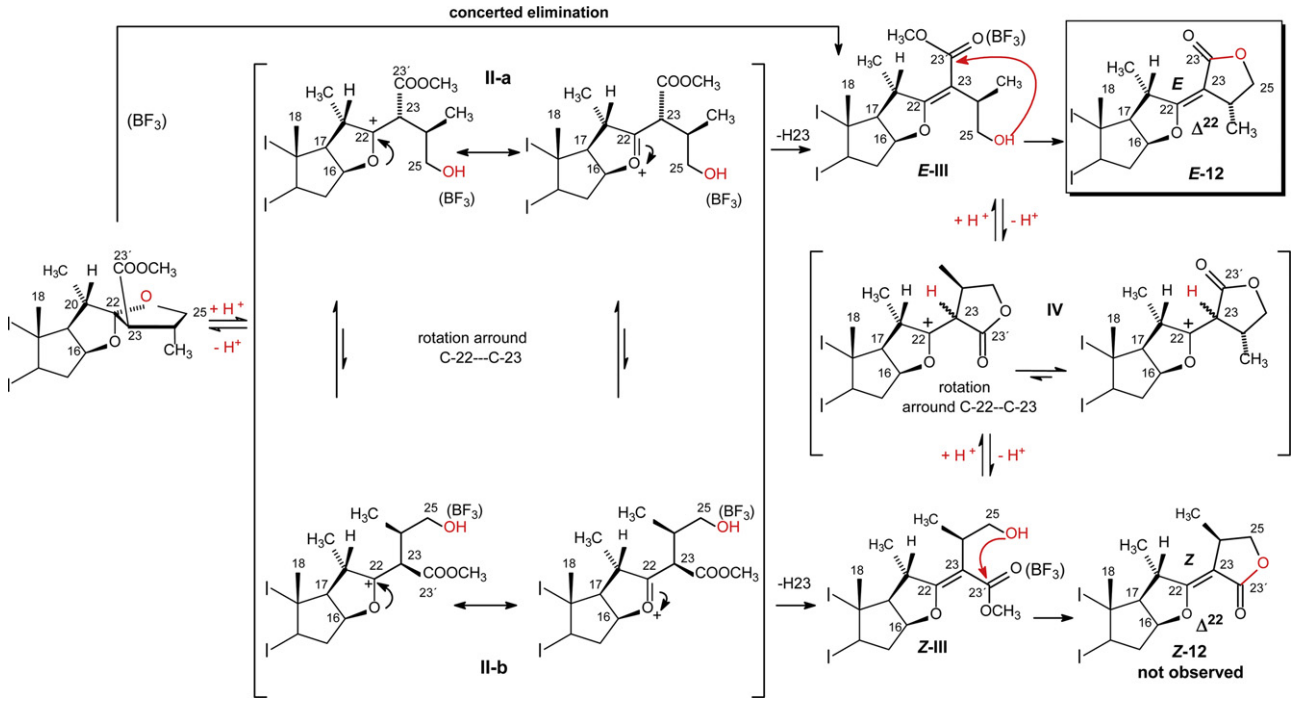
Acetylation of **10** following the standard Ac_2O/pyridine procedure afforded the expected compound **11** that on treatment with $BF_3{\cdot}Et_2O$ in acetic acid at room temperature for 10 days underwent transformation to the unsaturated lactone ***E*-12** in 88% yield as the sole product (see Scheme 3).

In the case of ***E*-12**, the presence of the conjugated double bond is evidenced by two ^{13}C olefinic signals that were differentiated and assigned to C-22 and C-23 with the aid of a HETCOR experiment (J fixed to 9 Hz) that corroborated the proposed unsaturated ester structure. In addition, the doublet multiplicity of H-21 discards the regioisomer bearing a double bond between C-20 and C-22 in which the absence of H-20 would results in a singlet multiplicity for H-21. The presence of the cyclic side chain in ***E*-12** can be corroborated by the different chemical shifts and the dd multiplicity of the diasterotopic pair of protons attached to C-25 as well as by their different NOE correlations (H-25 *Pro-S* ↔ H-24) and (H-25 *Pro-R* ↔ H-24′). The observed H-24′ ↔ H-16α NOE effect, only possible in the *E*-isomer, accounts for the proposed geometry of the double bond between C-22 and C-23 (see Fig. 3).

Coordination of BF_3 to O-25 results in the cleavage of the F-ring leading to the oxacarbenium intermediary **II**. Subsequent loss of a H-23 may produce the furostenic intermediaries ***E*-III** and ***Z*-III** that on catalysed transesterification undergo the lactone ring closure. The observed regioselectivity can be explained by the preference for the loss of H-23 over H-20 that is clearly justified by



Scheme 3.



Scheme 4.

the fact that it produces the more stable α,β-unsaturated ester in the Δ^{22} regioisomer. In addition, subtraction of H-20 that would lead to the $\mathbf{\Delta^{20(22)}}$ regioisomer is hindered by the presence of the axial CH_3-18. The exclusive occurrence of *E*-isomer can be justified by either the conformational preference for the oxacarbenium **II-a** that leads to ***E*-III** or by acid catalysed ***Z*-12** → ***E*-12** isomerization through the protonated intermediary **IV** that, on rotation around the bond between C-22 and C-23, reliefs the repulsion between the electronic pairs of O-16 and O-23′ in the *Z*-isomer. Nevertheless, the exclusive production of *E*-isomer may be better explained by a single-step elimination process in which the cleavage of the C-22 O-16 bond and loss of H-23 are concerted (see Scheme 4).

Treatment of **11** with acetic anhydride and $BF_3{\cdot}Et_2O$ at room temperature for 45 min produced extensive decomposition of the starting material affording a complex mixture from which two compounds were isolated and identified as the diacetylated cyclic enol ether **13** and the diacetylated furostene ***E*-14** (see Scheme 5).

As in ***E*-12**, in the endocyclic enol ether **13** the assignment of the observed olefinic signals to the unsaturated double bond between C-22 and C-23 is corroborated by the doublet multiplicity of H-21 that discards the regioisomer bearing a double bond between C-20 and C-22. Once more, the different chemical shifts and the *dd* multiplicity of the diasterotopic pair of protons attached to C-25, as well as their different NOE correlations (H-25 *Pro-S* ↔ H-24) and (H-25 *Pro-R* ↔ H-24′) allow their identification and corroborate the cyclic nature of the side chain in **13** (see Fig. 4).

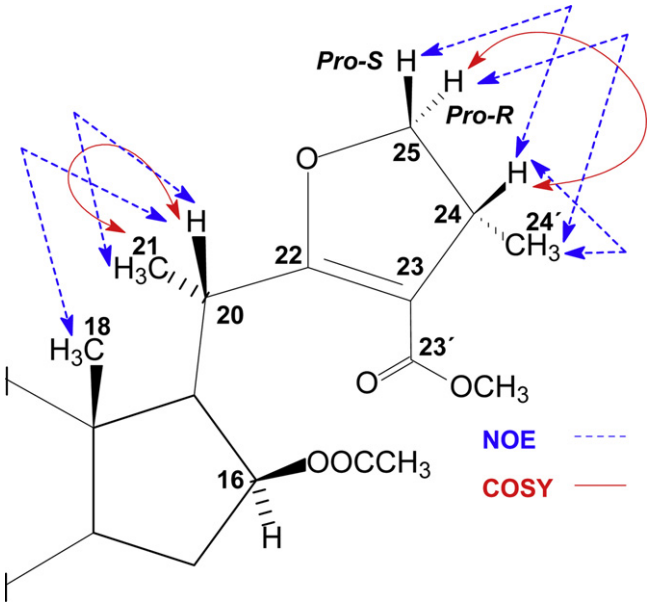
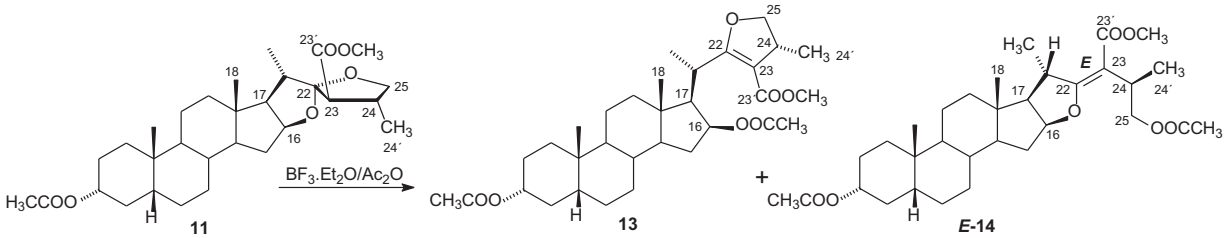


Fig. 4. Selected NOE and COSY correlations in the side chain of compound **13**.



Scheme 5.

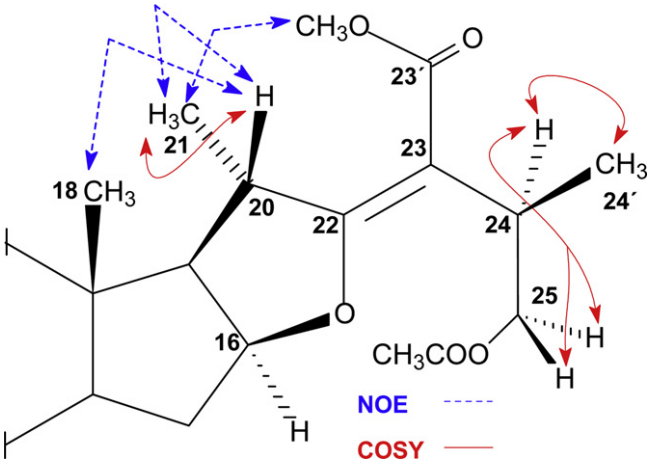


Fig. 5. Selected NOE and COSY correlations in the side chain of compound ***E*-14**.

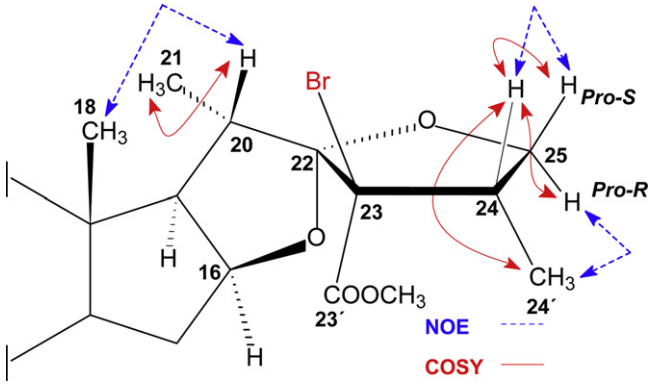


Fig. 6. Selected NOE and COSY correlations in the side chain of compound **23*R*-15**.

As in the preceding compounds ***E*-12** and **13**, in the diacetylated furostene ***E*-14** the two olefinic ^{13}C signals assigned to the double bond between C-22 and C-23 and the doublet multiplicity of H-21 that discards the Δ^{20-22} regioisomer, account for the structure bearing the exocyclic double bond. The observed $OCH_3 \leftrightarrow$ H-21 NOE effect indicates proximity of these two groups only possible in the *E*-isomer and accounts for the proposed *E*-geometry of the obtained compound (see Fig. 5).

Activation of acetic anhydride by BF_3 produced the acetylation of O-16 that results in the cleavage of the bond between O-16 and C-22 leading to the oxacarbenium **V**, which looses a proton from C-23 to produce the endocyclic enol ether **13** (see Scheme 6). The observed regioselectivity can be justified in terms of the preference for the loss of H-23 as explained above.

Similarly, activated acetic anhydride acetylates O-25 to produce the cleavage of the bond between O-25 and C-22 leading to the oxacarbenium **VI** that loses a proton from C-23 producing the observed diacetylated furostene ***E*-14**. Again steric hindrance, that prevents the subtraction of H-20 in concert with the fact that loss of H-23 leads to the more stable α,β-unsaturated system, clearly justify the exclusive occurrence of the Δ^{22}-regioisomer ***E*-14**. The stereoselective production of ***E*-14** can be explained by the conformational preference of the intermediate **VI**; but once again, one-step elimination to the observed diacetylated furostene ***E*-14** seems to be a better explanation (see Scheme 7).

3.2. Reactions under Brønsted acid catalysis

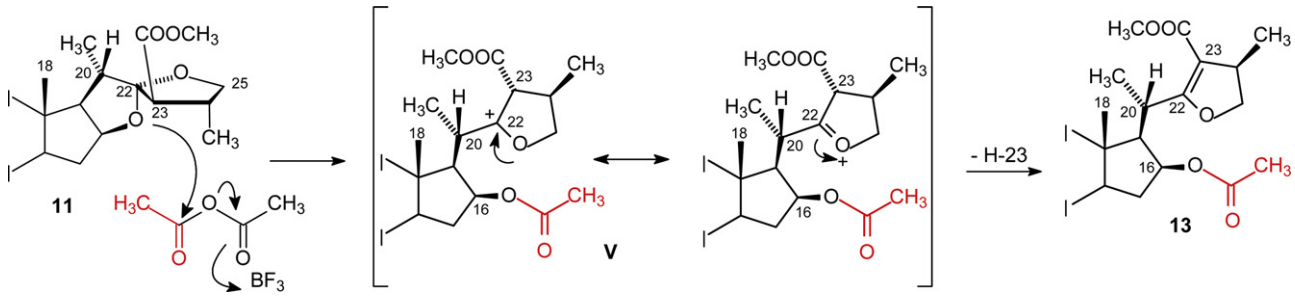
Treatment of **11** with $PyHBr{\cdot}Br_2$ in acetic acid at 50 °C afforded a 1/2.4 mixture of the diasteromeric 23-brominated compounds **23*R*-15** and **23*S*-15** that were identified after chromatographic separation (see Scheme 8).

The absence of the signal corresponding to H-23 in the ^1H spectrum of brominated furospirostane **23*R*-15** indicates the quaternary nature of C-23. All the observed ^1H and ^{13}C signals and 2D correlations corroborate the integrity of the furospirostane side chain (see Fig. 6), but give no evidence on the orientation of the substituents attached to C-23.

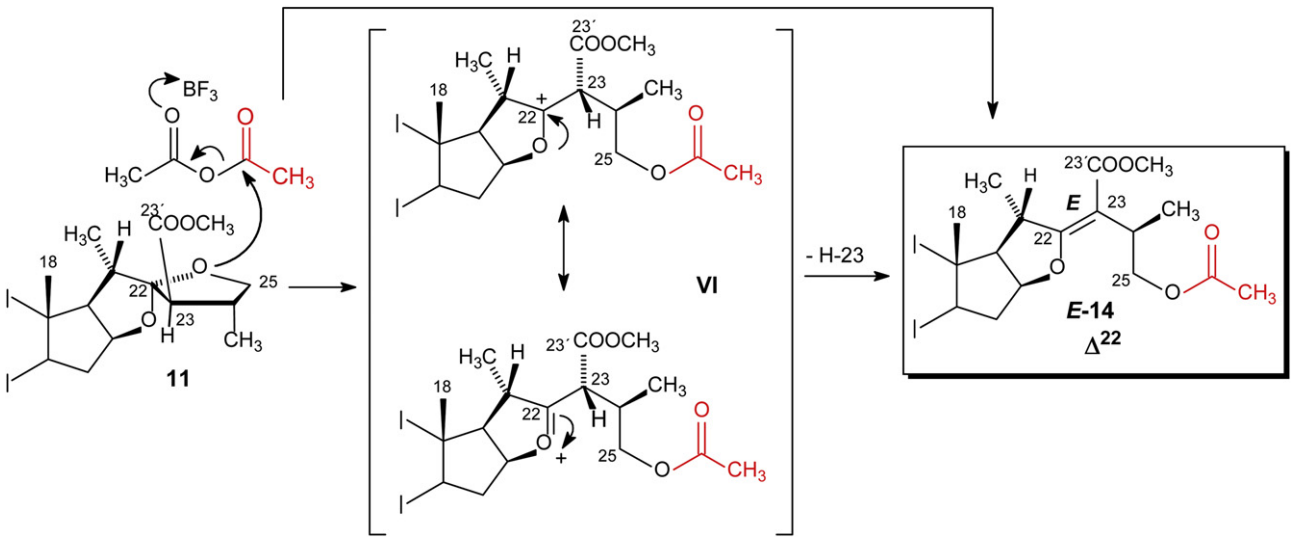
3.3. Crystal structure of 23R-15

The impossibility to assign the C-23 configuration employing NMR prompted us to obtain crystals of **23*R*-15** suitable for X-rays diffraction studies. After several attempts at crystallization in different solvent systems we were glad to find that slow crystallization in hexane/ethyl acetate solution afforded an adequate crystal of **23*R*-15**. Fig. 7 displays the crystal structure of **23*R*-15**, showing that the bromine atom attached to C-23 is in *trans* orientation relative to the 24′methyl group, resulting in a 23*R* configuration.

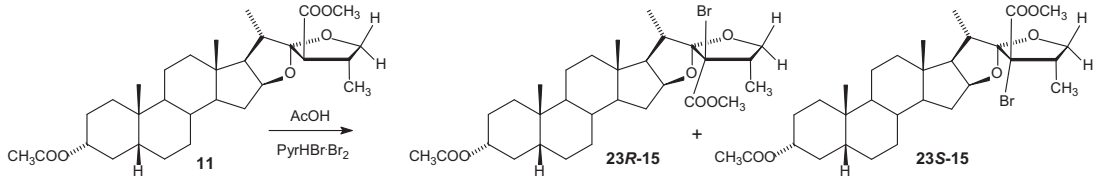
The presence of the equatorial α-acetoxy group attached to C-3 does disturb the chair conformation of ring A [puckering parameters [56] $Q = 0.555\ (3)$ Å, $\theta = 175.3\ (3)°$, $\varphi = 242\ (4)°$, if the calculation starts from C-1 to C-10 and proceeds in a clockwise direction]; all asymmetry parameters are less than 7.5 (3)° [57]. Rotational symmetry is dominant; a pseudo-C2 axis bisects the C-3–C-4 bond with asymmetry parameters ΔC_2(C-3–C-4) = 7.5 (3)° and ΔC_s(C3) = 4.9 (2)°. The average magnitude of the torsion angles is 54.5 (3)°; the A/B rings junctions are *cis*. Ring B assumes a chair conformation [puckering parameters $Q = 0.566\ (3)$ Å, $\theta = 4.2\ (3)°$, $\varphi = 243\ (4)°$, if the calculation starts from C-5 to C-10 and proceeds in a clockwise direction]; the B/C rings junctions are *trans*. Ring C also assumes an almost perfect chair conformation [puckering parameters $Q = 0.564$ (3) Å, $\theta = 7.5\ (3)°$, $\varphi = 249.6\ (19)°$; if the calculation starts from C-8 to C-14 and proceeds in a counterclockwise direction]; the C/D rings junctions are *trans*. The five-membered D ring has a C-13–C-14 twisted conformation with puckering parameters (C-13–C-17) $q2 = 0.458\ (2)$ Å and $\varphi 2 = 201.7\ (3)°$ [pseudorotation [58] and asymmetry parameters: $\Delta = 9$, $\tau_m = 46.5\ (2)$, $\Delta C_s(14) = 12.8$ (2) and $\Delta C_2(13,14) = 5.9\ (2)°$; the methyl groups at C-10 and C-13 are β in the steroid structure, additionally the tetrahydrofuran ring E is *cis*-fused to the cyclopentane ring D and the tetrahydrofuran ring E shows envelope O3-*exo*, [puckering parameters



Scheme 6.



Scheme 7.



Scheme 8

Scheme 8.

$q2 = 0.343\ (2)$ Å and $\varphi 2 = 183.0\ (4)^\circ$]. Finally in spite the presence of 23-methoxycarbonyl group and the bromide in C-23 and the methyl group and C-24, the five-membered tetrahydrofuran F-ring of has an envelope C-23-*exo* conformation [puckering parameters $q2 = 0.371(3)$ Å and $\varphi 2 = 245.7(4)^\circ$].

In the crystal structure, each molecule features pairs of C_{furan}–H···Br–C bonds to its neighbors related by the symmetry operations *x*, *y*, *z*. These van der Waals interactions lead to infinite ribbons of D motifs [59] run in the direction of the crystallographic b axis.

The NMR signals corresponding to the side chain of **23*S*-15**, that together with the observed 2D correlations, (see Fig. 8) corroborate the integrity of the furospirostane side chain in which, again, the absence of the signal of H-23 suggest the presence of an atom different than a hydrogen attached to this position. No direct proofs for the configuration at C-23 could be found in the NMR spectra.

Since in our hands compound **23*S*-15** resisted all attempts at crystallization, determination of the orientation of the substituent attached to C-23 employing X-ray diffraction was not possible. Nevertheless, the configuration of C-23 can be indirectly inferred by observation of the NOE effect between H-24 and H-21 only possible in a compound with the same configuration at C-22 as **23*R*-15**. Consequently both 22*R*, 23*R* and 22*R*, 23*S* epimers (*with inverted configuration at C-22*) can be discarded, leaving the 22*S*, 23*S* isomer as the unique possible structure for **23*S*-15**.

Considering that all the above cumulated evidence accounts for the more favored production of the ***E*-III** intermediate over the ***Z*-III** isomer, the occurrence and the diastereomeric relation of the brominated compounds **23*S*-15** and **23*R*-15** can be explained in terms of an electrophilic attack of the bromide to the furostenic intermediary ***E*-III**, in which the approach of bromine to the *Si* (upper) side of C-23, that leads to **23*S*-15**, is less hindered than the approach to the *Re* (lower) side, hindered by the presence of the C-21 methyl group (see Scheme 9). A similar process involving ***Z*-III** would have produced the reverse **23*S*-15**/**23*R*-15** diastereomeric relation that was not observed.

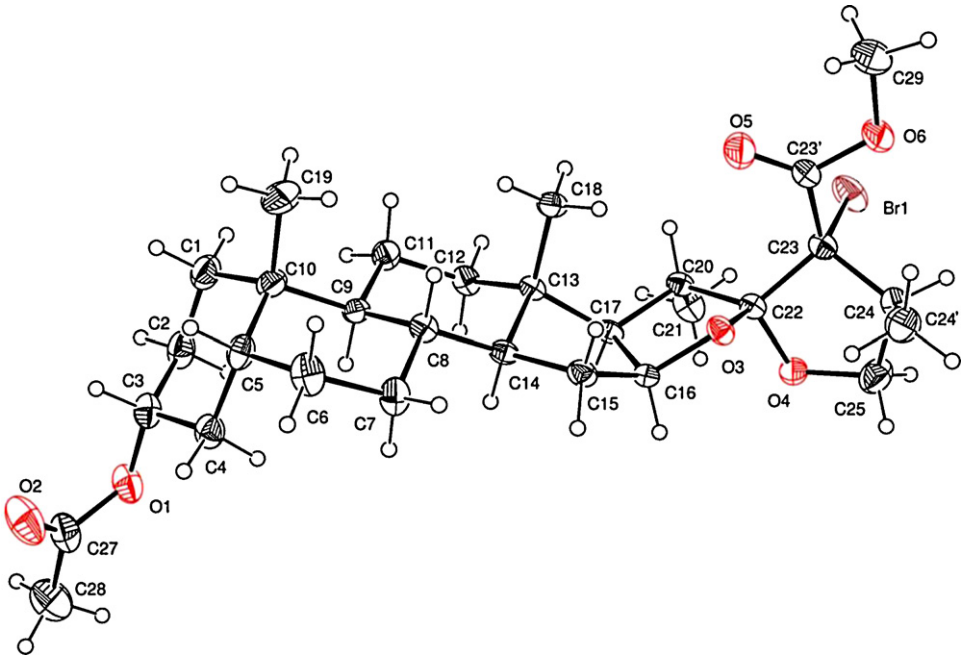
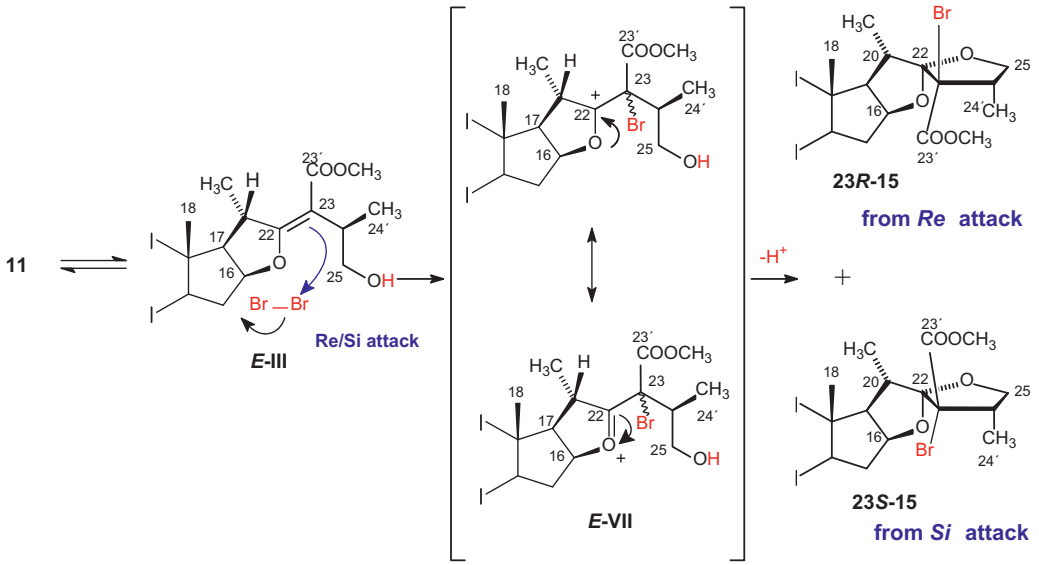


Fig. 7. Crystal structure of **23*R*-15** with the ellipsoids drawn at 30% of probability.



Scheme 9.

3.4. Deuteration experiments

Since all the above described results indicate the preference for the loss of H-23 that leads to the observed compounds, all through the corresponding α,β-unsaturated intermediary, we decided to study the deuteration profile of compound **11** to obtain evidences that allow to discard the occurrence of the $\Delta^{20(22)}$-furostenic intermediary.

Considering that deuteration at either C-20 or C-23 implies F-ring opening to produce either a $\Delta^{20(22)}$- or Δ^{22}-furostenic intermediary (see Scheme 10), the fact that the studied compound **11** in CD_3COOD solution did not show evidence of incorporation of deuterium even after 24 h (see Fig. 9, spectrum **A**) indicates that, at room temperature, the studied side chain is not prone to F-ring opening in neutral or even the acidic media provided by CD_3COOD.

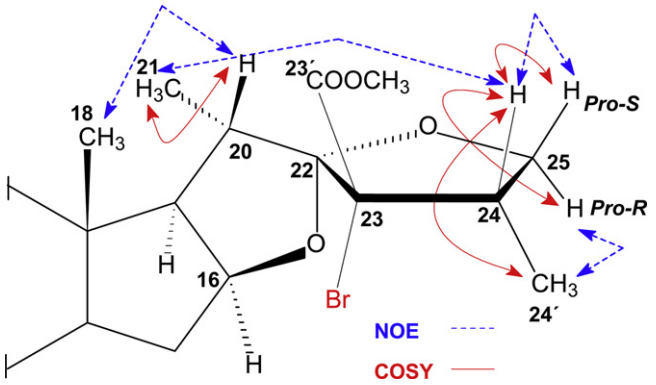
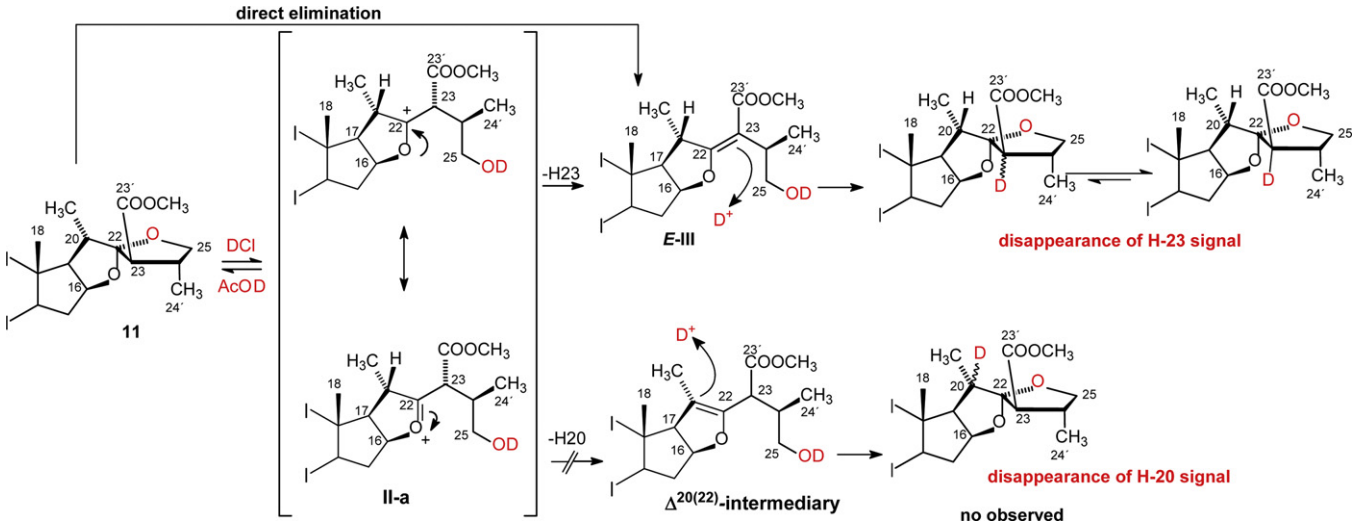


Fig. 8. Selected NOE and COSY correlations in the side chain of compound **23*S*-15**.



Scheme 10.

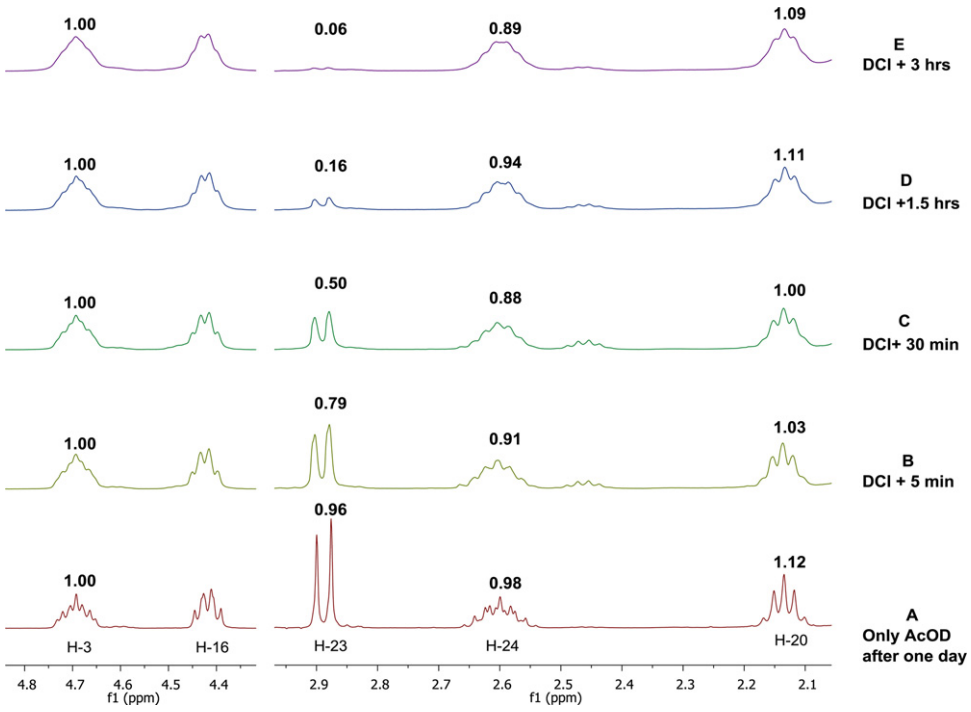


Fig. 9. Deuteration profile of compound **11** in CD3COOD/DCl.

Addition of a 35% DCl solution in D_2O (99% isotopic purity) resulted in slow incorporation of deuterium at C-23 as the disappearance of the H-23 signal indicated. The fact that after 24 h no variation in the H-20 signal was observed, clearly allows us to discard the occurrence of the $\Delta^{20(22)}$-intermediary, (see Fig. 9 spectra **B** to **E**). Slow precipitation of the studied compound explains the observed loss of resolution in the ^{1}H signals of the obtained spectra.

4. Conclusion

The obtained results demonstrate that under either Bronsted or Lewis catalysis, the furospirostanic side chain is opened to produce only the Δ^{22}-furostenic intermediary. Moreover, the outcome of all the studied reactions suggests that both reversible and irreversible F-ring opening involve the production of the *E* geometrical isomer of the Δ^{22}-furostenic intermediate.

The deuteration experiments indicate that, contrary to spirostanic sapogenins in which the order of incorporation of deuterium is C-23 (through a Δ^{22}-intermediary) $\gg$ C-20 (through a $\Delta^{20(22)}$ intermediary) $\ggg$ C-25 (through an iso-reaction) [60], at room temperature the studied side chain exclusively incorporates deuterium to C-23. Another interesting fact is the absence of incorporation of deuterium even in the acidic media provided by D_3CCOOD; this fact accounts for the resistance of the studied fragment to F-ring to opening, either through a concerted elimination process or through an oxacarbenium ion around C-22.

Further experiments directed to study the kinetics of the deuteration and the biological activity as well as the reactivity and capability to produce oxacarbeniums ions of this, and other compounds bearing furospirostanic side chains are planned. These studies may contribute to explain both the biological and chemical properties of this family of compounds. In addition, studies on

the reactivity of modified furospirostanic side chains may serve as a model for the reactivity of the 1,6-dioxaspiro[4.4]nonane moiety present not only in furospirostanes, but also in other naturally occurring spiroketals.

Acknowledgments

The authors thank to the *Dirección General de Asuntos del Personal Académico* (DGAPA-UNAM) for financial support via project IN221911-3 and CONACyT for scholarship granted to MM-A. Thanks are due to Nuria Esturau Escofet, Georgina Duarte Lisci and Victor Lemus Neri (USAI-UNAM) for recording NMR and MS spectra and Elemental Analysis. We are indebted to Dr. A. J. Boulton for his useful comments and for correcting the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.steroids.2011.04.006.

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