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5,10:13,14-Disecosteroids: novel modified steroids containing 10- and 9-membered rings

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ABSTRACT

In this paper a synthetic pathway to the modified 5,10:13,14-bisfragmentation cholestane derivatives **8**–**14** is described. The synthesis involves introduction of the 5α- and 14α-hydroxyl groups in the cholestane molecule and subsequent cleavage of the C(5)–C(10) bond in 5α,14α-dihydroxycholestan-3β-yl acetate (**4**) with the HgO/I_2 reagent and the C(13)–C(14) bond in the stereoisomeric 14α-hydroxy-5,10-secosteroids **5** and **6** with the $Pb(OAc)_4/I_2$ reagent. Complete and unambiguous ^1H and ^{13}C NMR resonance assignments of the obtained secosteroids, as well as the solution conformations of their 10- and 9-membered rings were determined by extensive analysis of 1D and 2D NMR spectral data. The structures and the solid-state conformations of 5,10-secosteroids **5**–**7** were confirmed by X-ray analysis. All diseco-compounds have a novel 5,10:13,14-disecocholestane skeleton.

1. Introduction

Steroids are widespread compounds in almost all living organisms expressing various types of biological activity. It has been shown that certain structural modifications of the rigid tetracyclic steroidal skeleton by bond cleavage induced a change of biological response, giving compounds with pronounced biological activity (i.e., 5,10-secosteroids as inhibitors steroid hormone biosynthesis[1]). Many natural products containing 13,14-secosteroid skeleton have been found to exhibit diverse biological activities, e.g., GST inhibitory[2a] and strong cytotoxic activity.[2b,c] Until now, only a few research groups have reported studies on the synthesis of 13,14-secosteroids.[3]

The synthesis of 5,10-,[4] 13,14-,[5] and B-nor-5,10-secosteroids,[6] as well as the conformational studies of their rings have been the focus of our continuing interest in transformation of steroid compounds to products with modified carbon skeleton. These secosteroids contain medium-sized rings incorporated into the steroid nucleus. It is known that alkoxy radicals obtained by the oxidation of 5- and 14-hydroxysteroids such as **I**–**III** with lead tetracetate (LTA) and hypoiodite-forming reagents, readily undergo β-fragmentation of the C(5)–C(10) and C(13)–C(14) bonds to give, via C(10) and C(13) radical intermediates, the diastereoisomeric (*Z*)- and (*E*)-1(10)-unsaturated-5-oxo-5,10-secosteroids **IV** and **V** (from **Ia**,**b**), the diastereoisomeric (*Z*)- and (*E*)-1(10)-unsaturated B-nor-5,10-secosteroids **XI** and **XII** and their 10(19)-unsaturated isomer **XIII** (from **IIIa**,**b**), the diastereoisomeric (*Z*)- and (*E*)-12-unsaturated-14-oxo-13,14-secosteroids **VI** and **VII** and their isomeric 13(18)-unsaturated-13,14-seco-14-ketone **VIII** (from **II**), as the primary products. In addition, 12,13-epoxy-13,14-secosteroid **IX** and 14β,22-ether **X** (from **II**), the stereoisomeric 5,10-acetals **XIV** and **XV** (from **IIIa**,**b**), and 5β,19-oxetane **XVI** (from **IIIb**) are obtained. These studies have shown that the fate of the nine-membered ring C(10) radical in B-nor steroids **IIIa**,**b**[6] is considerably different from that of the corresponding 10-membered ring analog in 5-hydroxy steroids **Ia**,**b**[4] and from the nine-membered ring C(13) radical in 14α-hydroxy steroid **II**[4] (Scheme 1).

To the best of our knowledge, the synthesis of 5,10:13,14-disecosteroids has not been reported until now. On the basis of the previous findings that 5,10- and 13,14-secosteroids can readily be prepared by oxidative fragmentation of the C(5)–C(10) and C(13)–C(14) bonds in 5- and 14-hydroxy steroids,[4–6] it was expected that similar oxidations of 5α,14α-diol of type **4** could result in a simultaneous β-fragmentation of the C(5)–C(10) and C(13)–C(14) bonds to afford

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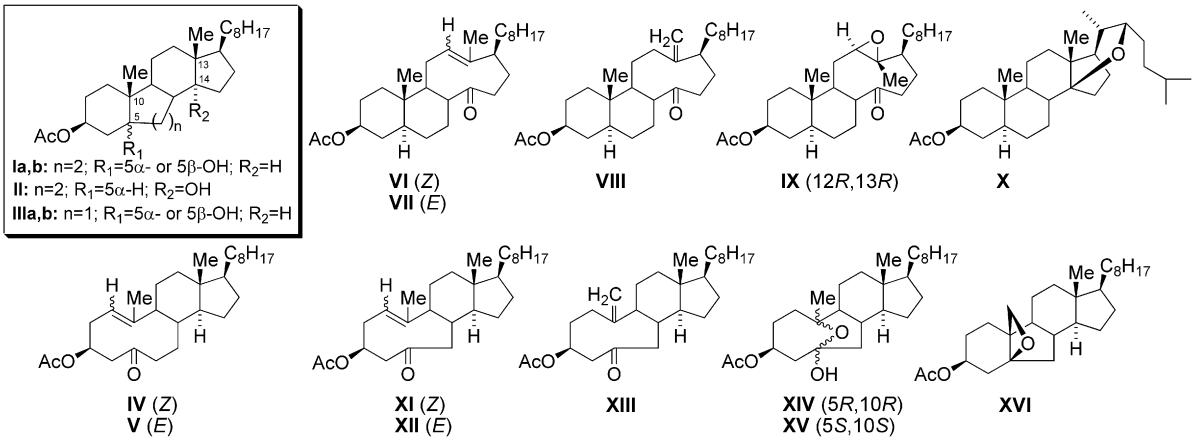


Scheme 1.

5,10:13,14-diseco steroidal derivatives, containing fused 10- and 9-membered rings. The aim of the present investigation was to prepare and study the behavior of the 5α,14α-dihydroxy cholestane derivative **4** under the conditions similar to those previously applied to the monohydroxy steroid derivatives.[5,6a] Starting from **4**, we obtained 5,10-seco- and 5,10:13,14-disecocholestane derivatives. Their complete and unambiguous ^{1}H and ^{13}C resonance assignments were determined with standard procedures using 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, TOCSY, HSQC, HMBC, and NOESY) NMR data. The NOESY spectra allowed us to distinguish the preferred solution conformations of medium-sized rings from the other possible conformations. The examination of Dreiding molecular models suggests that the mobility of the 10- and 9-membered rings of a targeted 5,10:13,14-disecosteroids is partly constrained by the presence of the alkyl chain, the 3β-OAc function, the olefinic and carbonyl groups, as well as by the ring junction at C-8 and C-9. Taking into account some conformational effects in 10- and 9-membered rings, such as the inversion of the orientation of the olefinic methyl and carbonyl groups, the number of stable conformers is limited to the number of combinations of their orientations.[†]

2. Results and discussion

2.1. Synthesis of 5α,14α-diol 4

The synthesis leading to the required 5α,14α-diol **4** is shown in Scheme 2. The starting point consisted in the preparation of the known cholest-8(14)-ene-3β,5α-diol 3-acetate **1** in ~26% yield, over six steps, using the described procedures (the allylic bromination–dehydrobromination of cholesteryl acetate[7]; the preparation of the Δ^{6}-unsaturated-5α,8α-peroxide[8]; the catalytic hydrogenation of the unsaturated peroxide, followed by reductive opening of the epidioxy bridge with simultaneous elimination of the 8-OH group[9]; the catalytic isomerization of the Δ^{7}-double bond to the 8(14)-position[7]). Isomerization of the $\Delta^{8(14)}$-double bond in compound **1** to the Δ^{14}-position with HCl in dry $CHCl_3$ gave compound **2** in 65% yield. Stereoselective epoxidation of the Δ^{14}-double bond of compound **2** with *m*-chloroperbenzoic acid afforded the corresponding 14α,15α-epoxide **3** in nearly quantitative yield. Stereoselective reduction of the epoxide **3** with $LiAlH_4$, followed by re-acetylation of the resulting 3β,5α,14α-triol with Ac_2O in pyridine, gave the targeted 3β-acetoxy-5α,14α-diol **4** in 65% yield.

2.2. Oxidative β-fragmentation of the C(5)–C(10) bond in 5α,14α-diol 4

Our attempt to obtain 5,10:13,14-disecosteroids by bis-fragmentation of the C(5)–C(10) and C(13)–C(14) bonds in 5α,14α-dihydroxy derivative **4**, under the LTA hypoiodite conditions, was unsuccessful. The LTA version of the hypoiodite reaction of **4** was carried out with a large excess of oxidant, in C_6H_6 solution, by irradiation with a 800 W lamp at 100 V, at room temperature (Scheme 2), resulting only in a complex mixture of diseco-compounds, inseparable by chromatographic methods (procedure has not been given in Experimental part). It was found that, in contrast to 5-hydroxy steroids,[4,6a] procedures using HgO/I_2 as oxidizing agent were inefficient to induce β-fragmentation of the C(13)–C(14) bond in 14α-hydroxy steroid.[5] Therefore, we anticipated that reaction of 5α,14α-diol **4** with HgO/I_2 would lead to a mixture of 14α-hydroxy-5,10-mono-fragmentation products, which could be converted into 5,10:13,14-di-fragmentation products by treatment with LTA/I_2.

The HgO/I_2 oxidation of **4** was carried out with an excess of oxidant in CCl_4 solution, by irradiation with an 800 W lamp at 140 V, at room temperature, for 4 h (Scheme 2). The resulting mixture was separated by flash column chromatography (FCC), affording the stereoisomeric (*Z*)- and (*E*)-1(10)-unsaturated-5,10-secosteroidal 5-ketones **5** and **6** (10 and 53% yield, respectively), and the (1*R*,10*R*)-epoxide **7**[‡] (6%) derived from (*E*)-1(10)-unsaturated-5,10-seco-ketone **6** (Scheme 2).

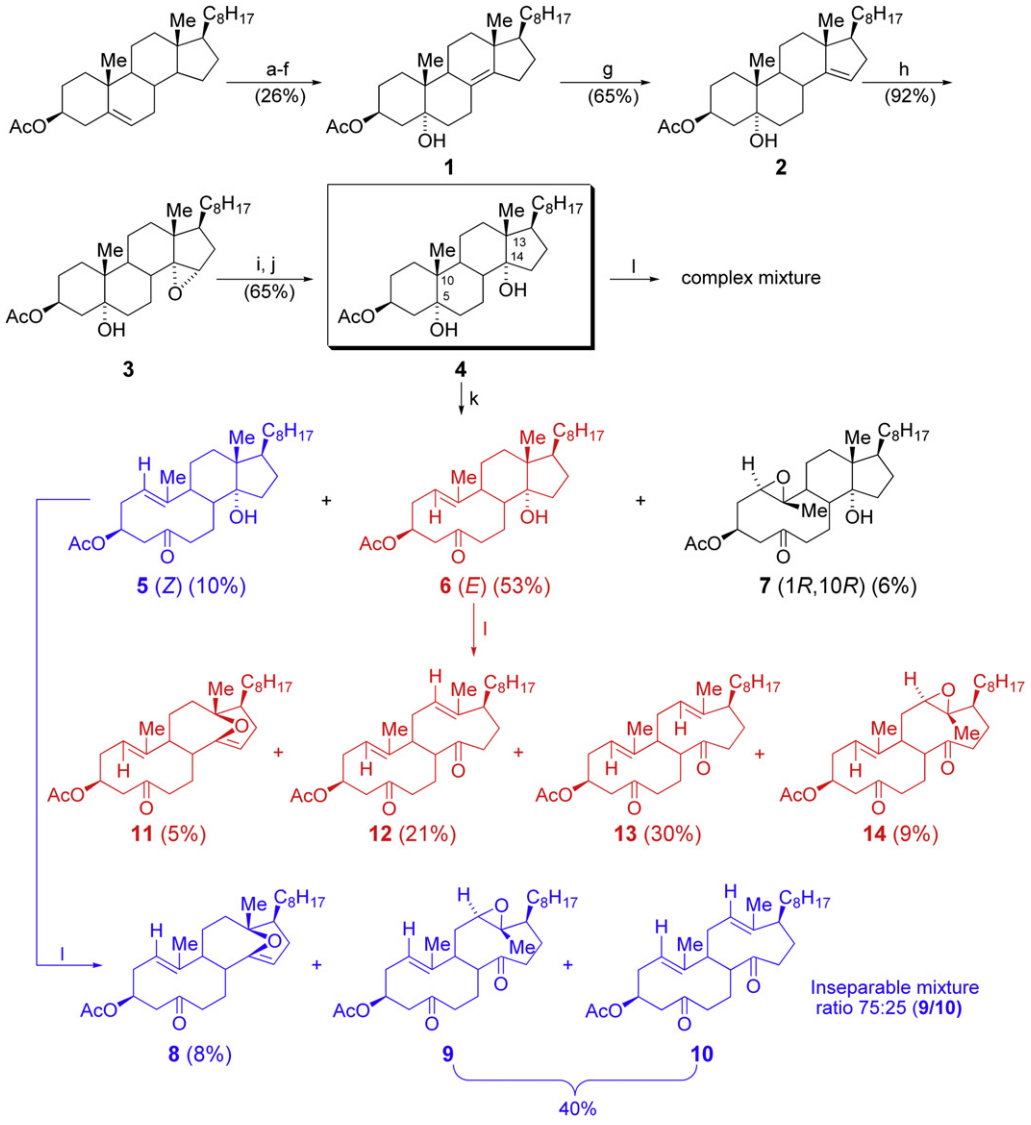
2.3. Structures and conformations of secosteroids 5–7

The structures **5**–**7** were deduced from their analytical and spectral data (IR, MS, and NMR). The identification of compounds **5**–**7** was based on extensive analysis of their NMR data (Table 1) and comparison of the NMR data with those of the related 5,10-secosteroids.[4c–e] In particular, the HMBC correlations observed for compounds **5**–**7** (H_3-19 with C-1, C-9, and C-10 and not with C-5) established the presence of a 5,10-secosteroid moiety.

The appearance of the 5-oxo group (IR: 1699 cm^{-1} for all three; ^{13}C NMR: 213.4s (**5**), 205.2s (**6**), 208.3s (**7**)), along with the disappearance of the C(5)–OH group from diol **4** (^{13}C NMR: 75.6s) are in accordance with 5,10-seco-structures **5**, **6**, and **7**. The ^{1}H NMR spectra of **5** and **6** show the downfield shift of the CH_3-19 signal at

[†] Regarding the relative orientation of the olefinic methyl and carbonyl groups, conformations of the 10- and 9-membered rings are named as αα, αβ, βα, and ββ.

[‡] An unusual epoxidation of the double bond in some steroid and 13,14-secosteroid derivatives was observed during their oxidation with hypoiodite HgO and LTA reagents (reactive species in these reactions is I_2O).[5]



Scheme 2. Reagents and conditions: (a) NBS, *n*-hexane, CCl_4, *hν*; (b) $NaHCO_3$, α-picoline, xylene, a, b (47%); (c) *hν*/O_2, C_6H_6/MeOH, eosin, 60%; (d) H_2/PtO_2, EtOAc; (e) Zn/AcOH, d, e (99%); (f) H_2/PtO_2, AcOH, 93%; (g) HCl/$CHCl_3$, −30 °C; (h) *m*-CPBA, CH_2Cl_2; (i) $LiAlH_4/Et_2O$; (j) Ac_2O/Py; (k) HgO/I_2, CCl_4, *hν*; (l) LTA/I_2, CCl_4, *hν*.

δ 1.56 and 1.46 ppm and a single vinyl proton at δ 5.32 and 4.94 ppm, respectively. In addition, the endocyclic epoxy group in **7** was confirmed by the ^{13}C signals of two oxygenated carbons at δ 62.6 (d, C-1) and 63.8 (s, C-10) and an oxirane proton at δ 2.54 ppm (H-1), as well as by the ^2J and ^3J-HMBC correlations of the H_3-19 with C-10 and C-1, respectively. The assignment of stereochemistry of the endocyclic 1(10)-double bond in **5** and **6** and the configuration for the 1,10-epoxide of **7** have been determined from their NOESY spectra and from ^{13}C NMR chemical shifts of C-9 and C-19. The lowfield double doublet at 5.32 ppm (olefinic H-1) in seco-ketone **5** showed NOESY cross-peak with the CH_3-19 singlet (δ 1.56 ppm) (Fig. 1) indicating the (*Z*)-configuration of $\Delta^{1(10)}$-double bond. The absence of the NOESY cross-peak between the vinylic H-1 (δ 4.94 ppm) and CH_3-19 (δ 1.46 ppm), as well as the presence of H-1/H-3 and CH_3-19/Hβ-2 NOESY correlations confirmed the (*E*)-geometry of 1,10-double bond in **6**. NOESY correlations between the H-1/H-3, H-1/H-9, CH_3-19/Hβ-2, CH_3-19/H-8 proton pairs in 1,10-epoxide **7** provided evidence for 1*R*,10*R*-configuration. The comparison of the ^{13}C NMR data for C-19 signal of secosteroids **5**, **6**, and **7** indicates that in (*E*)-seco-ketone **6** and *trans*-1,10-epoxide **7**, due to the shielding interaction of the β-hydrogen at C-2 with CH_3-19 group (γ-cis effect), C-19 signal is upfield shifted (δ 13.1 and 13.8 ppm, for **6** and **7**, respectively, characteristic of β-oriented CH_3-group) in respect to (*Z*)-isomer **5** (δ 18.6 ppm). The signal of C(9) of (*Z*)-5,10-seco-ketone **5** appears at much higher field (δ 36.6 ppm) (two γ-effects due to HαC-2 and HαC-6 bonds) comparing with the corresponding chemical shifts of seco-ketones **6** (δ 50.1 ppm) and **7** (δ 47.3 ppm), where these effects do not exist (Table 1, Fig. 1). The ^1H resonances of Hα-17, Hα-16, and Hα-12 protons in **5**–**7** (see Table 1) show large downfield shifts because of their 1,3-diaxial orientation to the 14α-hydroxyl group. Also, significant downfield chemical shift for Hα-6 at δ 2.69 ppm is observed in **5**, due to the spatial proximity of 14α-hydroxyl group in the (*Z*)-1(10)-configured cyclodecen-5-one ring. These chemical shifts are highlighted, because they are important in the structural analysis of other obtained secosteroids.

One set of NMR resonances of 5,10-secosteroids **5** and **7** suggests only one conformational form of their 10-membered ring in C_6D_6 solution, at room temperature (Fig. 1). The conformation of the 10-membered ring in (*Z*)-5,10-secosteroid **5** and epoxide **7** was established by the NOESY correlations (CH_3-19 with H-1 and H-8; Hβ-4 with H-1 and H-7; as well as Hα-6 with OH, for **5**; H-1 with H-3, H-7, and H-9, and CH_3-19 with Hβ-2, Hβ-4, Hβ-6β, and H-8, for **7**; indicated by arrows on Fig. 1). However, the NMR spectra of the (*E*)-

Table 1
^{1}H and ^{13}C NMR chemical shifts and selected NOESY data of **5**–**7** (δ, mult., J in Hz)

No.	**5**			**6**[a] (85:15)[b]			**7**		
	δ_H	δ_C	NOESY	δ_H	δ_C	NOESY	δ_H	δ_C	NOESY
1	5.32dd (4.5, 12.0)	121.9d	2β; 4β; 19	4.94dd (3.2, 12.0) [5.31t, 7.5]	124.3d [117.1d]	2α; 3; 9; 7	2.54br d (~10)	62.2d	2α; 3; 9; 7α
2	α: 2.85br t (~13) β: 2.13br d (~13)	29.1t	3; 9 1; 3	α: 2.41m β: 2.29br q (~12)	34.7t	3; 1 19	α: 2.36dd (3.5; 14.0) β: 1.64m	34.2t	1; 3 4β; 19
3	5.62br d (11)	70.9d	2α,β; 4α,β	5.72m [5.36m]	74.8d [71.8d]	1; 2α; 4α	5.36m	70.4d	1; 2α; 4α
4	α: 2.24br d (~16) β: 2.81br t (~16)	41.6t	3 1; 3; 7	α: 2.22br t (~12) β: 2.43br d (s~12)	48.1t	6β	α: 2.68m β: 2.74m	48.0t	3 19
5	—	213.4s		—	205.2s		—	208.3s	
6	α: 2.69br t (~13) β: 1.82br d (~13)	42.6t	OH	α: 2.01m β: 1.88m	42.7t	4β	α: 2.55m β: 2.73m	43.0t	19
7	α,β: 1.6m	25.6t	4β	1.58m	26.9t	1	α: 2.02m β: 1.78m	23.2t	9; 1
8	1.83m	39.9d	18; 19	1.44m	41.6d		1.69m	42.2	6β; 18; 19
9	2.77m	36.6d	2α; 11α; OH	2.38m	50.1d	1; 11α; OH	1.2m	47.3d	1; 7α; 11
10	—	142.2s		—	140.4s		—	63.8s	
11	α: 1.14m β: 1.52m	26.9t	9	α: 1.26m β: 1.97m	27.7t	9	α,β: 1.68m	23.2t	
12	α: 1.84m β: 1.48m	32.7t	17	α: 1.94m β: 1.54m	32.2t		α: 1.70m; β: 1.64m	31.4t	
13	—	48.3s		—	47.3s		—	46.8s	
14	—	86.1s		—	85.7s		—	86.6s	
15	α: 1.59m β: 1.38m	33.4t	OH	α: 1.40m β: 1.31m	34.7t		α,β: 1.56m	34.3t	
16	α: 2.22m β: 1.33m	28.2t		α: 2.21m β: 1.41m	23.9t		α: 1.96m; β: 1.32m	26.9t	
17	2.11m	51.4d	OH; 12α	1.97m	51.8d		1.75m	51.1d	
18	0.75s	16.6q	8	0.72s [0.81s]	15.9q [16.1q]	19	0.85s	15.8q	8
19	1.56s	18.6q	1; 8	1.46s [1.78s]	13.1q [19.9q]	2β; 8	1.38s	13.8q	2β; 4β/6β; 8β
20	1.55m	36.6d		1.43m	36.6d		1.41m	35.9d	
21	0.96d	19.3q		0.96s	19.2q		0.89s	18.8q	
22	1.53m/1.19m	37.2t		1.47m/1.12m	37.2t		1.36m/1.01m	36.4t	
23	1.55m/1.28m	24.8t		1.46m/1.23m	25.0t		1.36m/1.14m	24.2t	
24	1.25m	40.4t		1.23m	40.3t		1.12m	39.7t	
25	1.68m	28.8d		1.56m	28.7d		1.52m	28.3d	
26	0.95d	23.2q		0.94d	23.0q		0.86d	22.8q	
27	0.94d	23.4q		0.94d	23.4q		0.87d	23.0q	
AcO	—	169.7s		—	169.4s		—	169.9s	
	1.71s	20.8q	3	1.72s	21.0q		2.04s	21.4q	
OH	2.41s		9; 6α/β; 17	1.05s		9			

[a] Values of some signals of the minor conformation are given in square brackets.
[b] The ratio of conformers based on the ^{1}H NMR data.

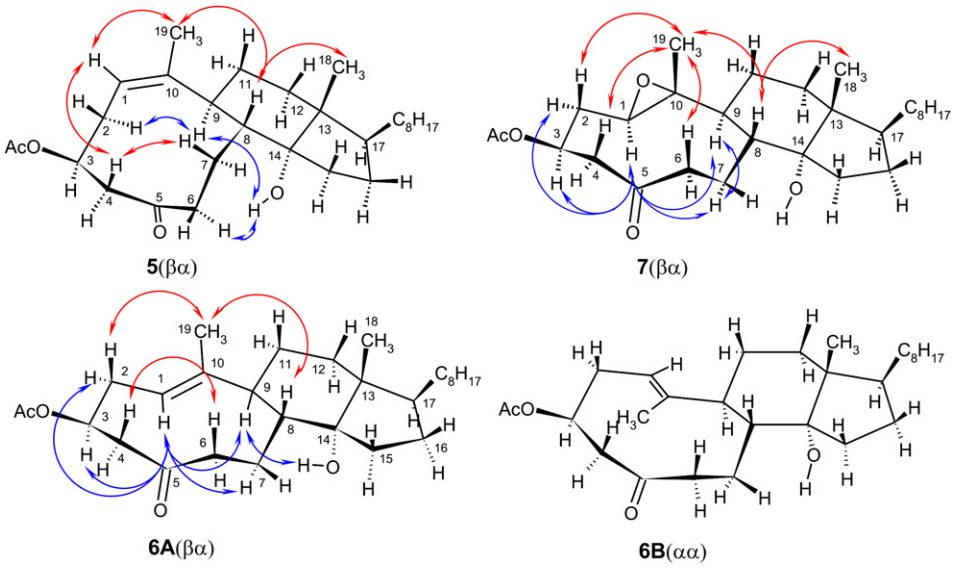


Figure 1. Solution conformations of **5**–**7** with the key NOESY correlations.

isomer **6** in C_6D_6 solution, at room temperature, indicate the presence of two conformers (**A**/**B**, 85:15) that differ in the orientation of the olefinic CH_3-19 group (Fig. 1). Typical NOESY correlations for the major conformer, **6A** (H-1 with Hα-3, Hα-7, and H-9; CH_3-19 with Hβ-2 and H-8; Hβ-4 with Hβ-6), are very similar to those observed for epoxide **7**, suggesting a similar conformation of 10-membered ring in **6A** (Fig. 1). The sharp lines in ^{1}H NMR spectra of **5**–**7** indicate their rigid conformations.[§]

The ^{1}H/^{13}C resonances at δ 1.78/19.9 ppm assigned to the olefinic CH_3-19 group of the minor conformer **6B** are characteristic for a methyl group located on the α-side of the steroid skeleton. The α-orientation of the CH_3-19 in **6B** was corroborated on the basis of the H-3 and H-1 resonances. The H-3 signal is upfield shifted (δ 5.36 ppm) due to the anisotropy of the 1(10)-double bond, while significant downfield chemical shift of the H-1 proton (δ 5.31 ppm) is the consequence of the close proximity to the 3β-OAc, in comparison with those (δ 5.72 and 4.94 ppm, respectively) of **6A** (Table 1, Fig. 1).

The structures of secosteroids **5**–**7** deduced from their NMR data were confirmed by X-ray analysis (Fig. 2, see Table 4 in Experimental). Crystal analyses of **5**–**7** have shown that the olefinic CH_3-19 group and 5-oxo function of 10-membered ring are on the opposite side as represented in Figure 2. For structures of **5** and **7**, there are two independent molecules in the asymmetric part of the unit cell. These two molecules have very similar conformations, differing only in the orientation of the side chain end.

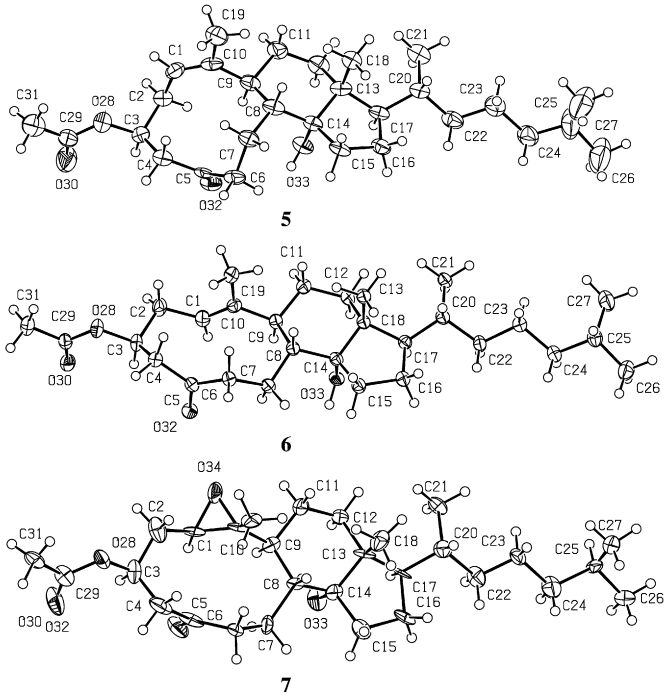


Figure 2. ORTEP plots of 5,10-secosteroids **5**–**7**.[10]

2.4. Oxidative β-fragmentation of the C(13)–C(14) bond in 14α-hydroxy-5,10-secosteroids 5 and 6

In the next step of the synthesis, the stereoisomeric (*Z*)- and (*E*)-14α-hydroxy-5,10-secosteroids **5** and **6** were oxidized with a large excess of LTA/I_2 reagent, in CCl_4 solution, by irradiation with a 800 W lamp at 140 V, at room temperature, for 5 and 2 h (conditions l, Scheme 2), respectively. Under these conditions, (*E*)-isomer **6** was fragmented to the both isomeric diseco-ketones, while (*Z*)-isomer **5**, due to the feasibility of hydrogen bonding between the hydroxyl group at C-14 and the C-5 ketone oxygen, reacted much more slowly and followed a more complex route, giving the mixture of products arising from the primary formed diseco-ketones.

The resulting mixture obtained from (*Z*)-seco-ketone **5** was separated by FCC, affording three products: the transannular (13*R*)-enol-ether with the β-oriented 13,14-epoxy bridge **8** (8%)[¶] as the minor product, and the inseparable mixture of two compounds that could be assigned as *trans*-12,13-oxirane **9** (originated from the targeted (*Z*,*E*)-5,10:13,14-disecocholesta-1(10),12-diene-5,14-dione, which was not isolated) and the expected (*Z*,*Z*)-5,10:13,14-disecocholesta-1(10),12-diene-5,14-dione **10** (**9**/**10**=75:25, ~40%) (Scheme 2).

Oxidative β-fragmentation of the C(13)–C(14) bond in 14α-hydroxy-(*E*)-5,10-seco-steroid **6** with LTA/I_2 afforded both expected stereoisomeric (*E*,*Z*)- and (*E*,*E*)-$\Delta^{1(10),12}$-unsaturated-5,10:13,14-diseco 5,14-diketones **12** and **13** (21 and 30%, respectively), the (13*R*)-enol-ether **11** (5%), and the (12*R*,13*R*)-epoxide **14** (9%) (Scheme 2).

2.5. Structures and conformations of disecosteroids 8–14

The structures of disecosteroids **8**–**14** were elucidated by detailed analysis of their NMR spectral data (Tables 2 and 3) and by comparison of the spectral data with those of the parent steroids **5** and **6** (Table 1).[‖] All compounds have a novel 5,10:13,14-disecocholestane type skeleton. The structural difference in C,D skeleton of secosteroids **8**–**14** in relation to the parent compounds **5** and **6** was supported by their HMBC spectra in which there is no correlation between H_3-18 and C-14. Additional insight into the conformational features of the major conformations (**A**) of these compounds has been obtained using NOESY connectivities. Due to the absence of NOESY cross-peaks for the minor conformations (**B**) of **11**–**14**, the NMR parameters for the characterization of their 10-membered rings were: ^{1}H/^{13}C chemical shifts of the olefinic HC-1, HC-3, the olefinic CH_3-19 group, and the shielding effect of the carbonyl groups on the olefinic protons.[††]

In the IR spectra of the (*Z*)- and (*E*)-stereoisomers **8** and **11** the 14α-hydroxyl band is missing, instead, new absorptions for an ether bond at 1019 and 1023 cm^{-1} and the olefinic bond at 1673 and 1678 cm^{-1}, respectively, are observed. The ESI-TOF–MS data of **8** and **11** established the molecular formula as $C_{29}H_{46}O_4$. The configuration of the 1(10)-olefinic double bond in the stereoisomeric pair **8** and **11** was evidenced on the basis of their NMR spectral data (Table 2), which were correlated to those observed for the parent 5,10-secosteroids **5** and **6** (Table 1). Most of the signals assigned to the carbon atoms forming C,D rings in compounds **8** and **11**, do not differ significantly (Table 2). Significant difference is observed for HC-9 (δ 2.23/46.8 ppm for **8** and δ 1.64/62.4 ppm for **11**), mainly as a result of the γ-gauche effects induced by the C-2 and C-6 in **8**. The ^{1}H NMR spectra show the singlet for CH_3-18 group at δ 1.22 ppm

[§] In the ^{1}H NMR spectra of compounds **5** and **6**, recorded at different temperatures (213–363 K) no signal splitting or collapsing (for **6**) was observed. Thereafter, ring dynamics either does not exist or it is too fast on the NMR time scale in the examined temperature range.

[¶] The formation of compounds **8** and **11** could be explained by assuming transannular attack of the O atom of the 14-oxo group at C-13 radical intermediate, followed by stabilization of thus obtained C-14 radical intermediate by elimination of an H atom from CH_2-15.

[‖] None of four new compounds (**11**–**14**) produced crystals suitable for X-ray analysis.

[††] The shielding influence of the C(5)-carbonyl group causes an upfield shift of the olefinic H-1 in the conformations where the carbonyl group and olefinic proton are parallel to each other.

Table 2
^{1}H and ^{13}C NMR chemical shifts and selected NOESY data of **8**, **11**, and **9** (δ, mult., J in Hz)

No.	**8**			**11**[a] (90:10)[b]			**9**[c]		
	δ_H	δ_C	NOESY	δ_H	δ_C	NOESY	δ_H	δ_C	NOESY
1	5.30dd (4.0; 12.5)	121.1d	19; 2β; 4β	4.84dd (2.5;12.5) [5.24t; 7.5]	124.2d [117.3d]	3; 2α; 9	5.17dd (4.5; 12.0) [5.25dd; 4.0;12.0]	121.3d [121.9d]	19; 2β; 4β [19]
2	α: 2.70ddd (3.5; 12.5; 16.0) β: 2.08br d (16)	29.6t	9	α: 2.43br d (12.5) β: 2.19q (12.5)	34.7t	1; 3 19	α: 2.77td (15.0; 3.0) β: 2.11br t (15.0) [2.13m; 2.75m]	29.3t [29.3t]	9
3	5.67m	72.3d	2β; 2α; 4α	5.73m [5.33m]	74.8d [71.9d]	2α; 4α; 1	5.61m [5.71m]	70.8d [71.3d]	2β; 2α; 4α
4	α: 2.37dd (5.0; 14.5) β: 2.67dd (10.5; 14.5)	42.9t	7β	α: 2.33dd (2.5; 13.5) β: 2.11td (12.5; 13.5)	48.1t	3 6β	α: 2.24dd (5.0; 15.0) β: 2.58dd (11.0; 16.0) [2.16m; 2.75m]	42.0t [40.6t]	7
5		210.4s			205.0s			210.9s	
6	α: 2.18br t (13) β: 1.99m	42.0t	15	α,β: 1.75–1.88m	43.0t		1.83m; 1.74m	42.2t	
7	α: 2.04m β: 1.56∼td (13.4; 3.0)	25.1t	4β	α: 2.23m β: 1.60m	25.0t	15 15	α,β: 1.56m	28.8t	
8	2.35br t (10)	44.3d	19	1.94m	45.9d		2.57m [2.70m]	58.6d [53.0d]	19
9	2.23dt (3.5; 10)	46.8d	2α; 11α	1.64m	62.4d	1; 11α	2.98br t (11.0) [3.09m]	37.6d [39.7d]	2α; 11α; 12α,
10		143.5s			141.2s			141.7s [142.3s]	
11	α: 1.10m β: 1.84dd (6.0; 12.0)	30.7t	9 19; 18	α: 1.12m β: 1.87m	29.8t	 18; 19	α: 1.64br d (14) β: 1.33m	33.5t	9; 12 18
12	α: 1.06m β: 1.96m	32.2t		α: 1.07m β: 2.00m	31.8t		2.51d (10.5) [5.31br d, (∼12]	64.0t [127.2d]	9; 11α; 15α;17 [18, 19]
13		80.5s			80.0s			62.4s	
14		157.2s			158.1s			211.8s [213.9s]	
15	5.58 dd (2.0; 7.0)	109.1d	6α; 16α,β	4.95dd (2.0; 6.5) [4.89br d; 5.0]	106.4d [105.8d]	7α,7β;16α,16β	α: 2.91br d (13.0) β: 1.92m	39.7t	12; 16α
16	β: 2.08m α: 1.98m	20.4t		β: 2.05m α: 1.93m	20.3t	15 15	α: 1.84m β: 2.14m	27.6t	17
17	1.40m	49.5d		1.41m	48.7d		0.65br s [2.10m]	52.0d [41.0d]	16α
18	1.22s	23.0q	11β	1.22s	23.4q	11β; 12β	1.04s [1.48s]	13.8q [19.4q]	11β; 21 [12]
19	1.62s	20.1q	1; 8; 11β	1.42s [1.60s]	12. 8q [19.4q]	2β; 4β; 8; 11β	1.34s [1.54s]	19.4q [21.2q]	1; 8 [1,12]
20	1.79m	35.0d		1.84m	35.0q		1.47m	35.7d	
21	0.89d (7.5)	19.8q		0.90d (6.5)	19.7q		1.17d (6.5) [0.75d]	18.3q [18.6q]	18
22	1.84m; 1.06m	39.5t		1.73m; 1.11m	39.6t		1.03m; 1.48m	34.9t	
23	1.25m; 1.44m	26.7t		1.26m; 1.45m	26.7t		1.21m; 1.40m	24.7t	
24	1.19m	40.2t		1.20m	40.2t		1.21m	40.2t	
25	1.52qui. (7)	28.6d		1.53qui. (7)	28.6d		1.56m	28.7d	
26	0.876d (7)	23.1q		0.879d (6.5)	23.1q		0.942d (7)	23.1q	
27	0.881d (6.5)	23.2q		0.886d (7)	23.2q		0.946d (6.5)	23.4q	
AcO	1.70s	21.2q 169.7s		1.69s	21.2q 169.4s		1.72s	21.2q 169.7s	

[a] Values of some signals of the minor conformation are given in square brackets.
[b] The ratio of conformers based on the ^{1}H NMR data.
[c] Values of some signals of the (*Z*,*Z*)-disecosteroid **10** are given in square brackets.

(for both compounds) and the lowfield double doublet for the olefinic proton (H-15) at δ 5.58 (for **8**) and 4.95 ppm (for **11**). Also, in the ^{13}C NMR spectra, CH_3-18, C-13, C-14, and C-15 signals appear at 23.0q, 80.5s, 157.2s, 109.1d (for **8**), and 23.4q, 80.0s, 158.1s, and 106.4d (for **11**) indicating a transannular 13,14-enol-ether structure for both compounds. Especially, the HMBC correlations of H-15 with C-14, C-17, C-8, and C-16, as well as H_3-18 with C-13, C-17, and C-12, but not with C-14, proved the existence of the 14-unsaturated-13,14-epoxy bridge in both stereoisomers. The (13*R*)-configuration was deduced from NOESY spectra: the key NOESY correlations between the olefinic H-15 and Hα-6 for **8**, as well as H-15 and H-7α,β for **11**, provided the evidence for β-oriented 13,14-epoxy bridge.

In the (*Z*)-isomer **8**, H-1 signal, due to the deshielding influence of the 3β-OAc, appears at lower field (δ 5.30 ppm), than that in the (*E*)-isomer **11** (δ 4.84 ppm), which is in the shielding region of the C(5)-oxo group. Similarly as in seco-ketones **5** and **6**, NMR studies showed that the cyclodecenone ring of (*Z*)-5,10-seco-1(10),14-unsaturated-13,14-epoxy derivative **8** exists in only one conformation, while the 10-membered ring of (*E*)-isomer **11** adopts two conformations, **A** and **B** (90:10) in solution (Table 2, Fig. 3).

Significant chemical shifts of HC-1 (δ 5.24/117.3 ppm), HC-3 (δ 5.33/71.9 ppm), and H_3C-19 (δ 1.60/19.4 ppm) in the minor conformation **11B** are characteristic for α-oriented, CH_3-19, and 5-oxo groups (Table 2, Fig. 3).

Disecosteroids **12** and **13** were found to possess the same (*E*)-cyclodec-1(10)-en-5-one moiety as the parent compound **6** and the stereoisomeric (*Z*)- and (*E*)-cyclonon-12-en-14-one rings, respectively. The ESI-TOF–MS of these derivatives suggested the same molecular formula of $C_{29}H_{46}O_4$. In the ^{1}H/^{13}C NMR spectra of both compounds (Table 3) the signal of the original angular CH_3-18 group (δ 0.72/15.9 ppm) is missing. Instead, new ^{1}H singlets/^{13}C quartets at δ 1.41/19.3 and 1.36/14.0 ppm appear for **12** and **13**,

Table 3
^{1}H and ^{13}C NMR chemical shifts and selected NOESY data of **12**–**14** (δ, mult., J in Hz)

No.	**12**[a] (70:30)[b]			**13**[a] (92:8)[b]			**14**[a] (92:8)[b]		
	δ_H	δ_C	NOESY	δ_H	δ_C	NOESY	δ_H	δ_C	NOESY
1	5.24t (7.5) [4.78br d; 10.0]	118.6d [125.2d]	H2β, H8, H12	4.88dd (2.5; 12.5) [5.18t; 7.3]	125.3d [118.3d]	H2α, H9, H3	4.84dd (2.0; 12.0) [5.07t; 7.5]	125.6d [118.5d]	H2α; H3; H9
2	α: 2.59m; β: 1.99m	33.1t	H19, H3	α: 2.43br d (12.0) β: 2.13q (12.5)	34.6t		α: 2.41br d (12) β: 2.09∼q (12.5)	34.6t	H1 H19
3	5.18m [5.67m]	71.8d [74.9d]	H19, H2α, H4α	5.69m [5.28m]	74.6d [71.7d]	H2α, H4α, H1	5.68m [5.22m]	74.5d [71.6d]	H1; H2α; H4α
4	α: 2.23dd (10.0; 13.5) β: 2.51dd (3.5; 13.5)	48.3t	H3	α: 2.32dd (2.5;13.5) β: 2.04t (12.0)	48.0t	H6	α: 2.31m β: 2.00m	48.0t	H6
5		204.9s			204.3s			204.3s	
6	β: 2.19m; α: 1.68m	40.2t		1.80br t (8.0)	43.0t	H8, H4β	1.75∼t (5.5)	42.8t	H19, H8
7	α: 2.01m; β: 1.52m	26.7t		α: 2.23m; β; 1.27m	26.1t		1.17m; 2.17m	25.9t	
8	3.11t (10.5)	51.4d	H1	2.23m	58.7d		2.27d (11.5)	58.8d	H19; H6
9	2.23m	50.4d	H19	2.23m	59.5d		2.23t (11.0)	53.0d	H1
10		143.7s			139.5s			138.7s	
11	α: 2.60m β: 1.50m	28.2t	H17	α: 1.72m β: 2.20m	33.6t		α: 1.55m β: 1.39m	31.3t	H19
12	5.36dd (4.0; 12.5) [5.42br d; 9.5]	124.9d [127.1d]	H18, H11β, H1	4.84br d (11.0) [4.75br d; 8.7]	129.9d [129.9d]	H9, H15α, H17, H11α	2.31s∼t (10)	64.8d	H9, H17, H15α
13		139.6s			139.6s			62.7s	
14		217.4s			208.9s			209.4s	
15	α: 2.07m β: 2.37m	41.4t		α: 1.82m; β: 1.69m	37.7t	H12	α: 1.96m; β: 1.65m	35.6t	
16	α: 2.33m β:1.08m	27.4t		α: 1.69m β: 2.01m	27.5		α: 1.67m β: 2.05m	25.6t	H18
17	2.44m	42.4d	H11α	1.56m	57.1d	H12	0.65td (11.5; 4.5)	52.2d	H12
18	1.41s [1.50s]	19.3q [19.5q]	H12 [H12]	1.36s	14.0q	H11β, H20	1.13s [1.15s]	14.3q [14.3q]	H11β, H21, H16β
19	1.55s [1.45s]	19.0q [14.3q]	H9, H3, H11β, H2α	1.30s [1.60s]	12.6q [19.8q]	H2β, H4β, H8, H6β, H11β	1.19s [1.50s]	12.5q [19.4q]	H8, H2β, H4β, H6
20	1.37m	35.1d		1.48m	34.1d		1.53m	35.5d	
21	0.77d (6.5)	18.7q		0.89d (6.5)	19.3q		1.20d (7)	18.3q	
22	1.49m; 1.05m	34.5t		1.50m; 1.06m	34.7t		1.47m; 1.11m	34.9t	
23	1.46m; 1.19m	24.7t		1.41m; 1.24m	24.8t		1.38m; 1.23m	24.4t	
24	1.18m	40.2t		1.22m	40.2t		1.21m	40.3t	
25	1.53m	28.6d		1.58m	28.8d		1.57m	28.8d	
26	0.928d (6.5)	23.1q		0.952d (6.5)	23.1q		0.949d (7)	23.4q	
27	0.938d (6.5)	23.4q		0.958d (6.5)	23.4q		0.955d (6.5)	23.2q	
AcO	1.72s	21.2q 170.1s		1.70s	21.1q 169.4s		1.71s	21.1q 169.4s	

[a] Values of some signals of the minor conformation are given in square brackets.
[b] The ratio of the conformers based on the ^{1}H NMR data.

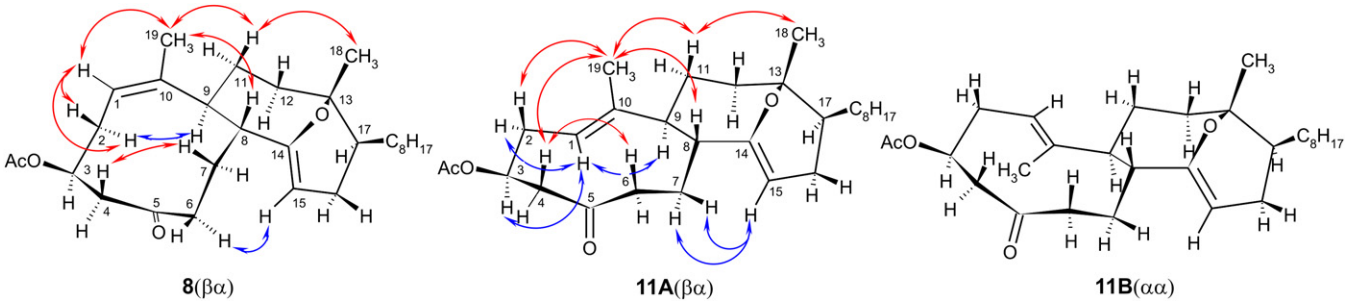


Figure 3. Solution conformations of **8** and **11** with the key NOESY correlations.

respectively, indicating that in these derivatives CH_3-18 group is attached to the olefinic Δ^{12}-double bond. Distinguishing between (*Z*)- and (*E*)-Δ^{12}-configuration in the stereoisomeric pair **12** and **13** was possible on the basis of the NMR chemical shifts of the H-12 (δ 5.36 and 4.84 ppm, for **12** and **13**, respectively) and C-18 (δ 19.3 and 14.0 ppm, for **12** and **13**, respectively), as well as by the presence (for **12**) and the absence (for **13**) of the NOESY correlations between olefinic H-12 proton and CH_3-18 group. The NMR signal of C-17 at δ 42.4 ppm for **12** compared with δ 57.1 ppm in **13** is upfield shifted, as a result of γ-gauche effect induced by the C-11, confirming the (*Z*)-stereochemistry of the 12,13-double bond of the nine-membered ring. The NMR data of diseco-ketone **12** indicated the coexistence of two conformers (**A** and **B**) in C_6D_6 solution, at room temperature (Fig. 4). The ratio (**A**/**B** 70:30) was determined from the peak areas of the olefinic H-1 and H-12 protons. The $^{1}H/^{13}C$ chemical shifts of the CH_3-19 group at δ 1.55/19.0 for **A** and 1.45/14.3 ppm for **B**, as well as HC-1 at 5.24/118.6 for **A** and 4.78/125.2 ppm for **B**, suggested the α- and β-orientation of the CH_3-19 group in **12A** and **12B**, respectively. Significant upfield chemical shift of H-3 in **12A** at δ 5.18 ppm regarding δ 5.67 ppm for **12B**, due to the anisotropy of the 1(10)-double bond, corroborated α-orientation of the CH_3-19 group in **12A**. The NOESY correlations observed for H-1/H-8, H-1/Hβ-2, and H-1/H-12, H_3-19/H-3, H_3-19/H-9, as well as H_3-18/H-12 provided the evidence that in **12A** the olefinic

protons (H-1and H-12) and CH_3-18 group are above the average plane of the molecule (β-oriented), while CH_3-19 and 5-oxo group are on the opposite side (α-oriented) (Fig. 4). The paramagnetic shift of the H-8 (δ 3.11 ppm) and Hα-16 (δ 2.33 ppm) can be attributed to deshielding by the α-oriented 14-oxo group, which is in

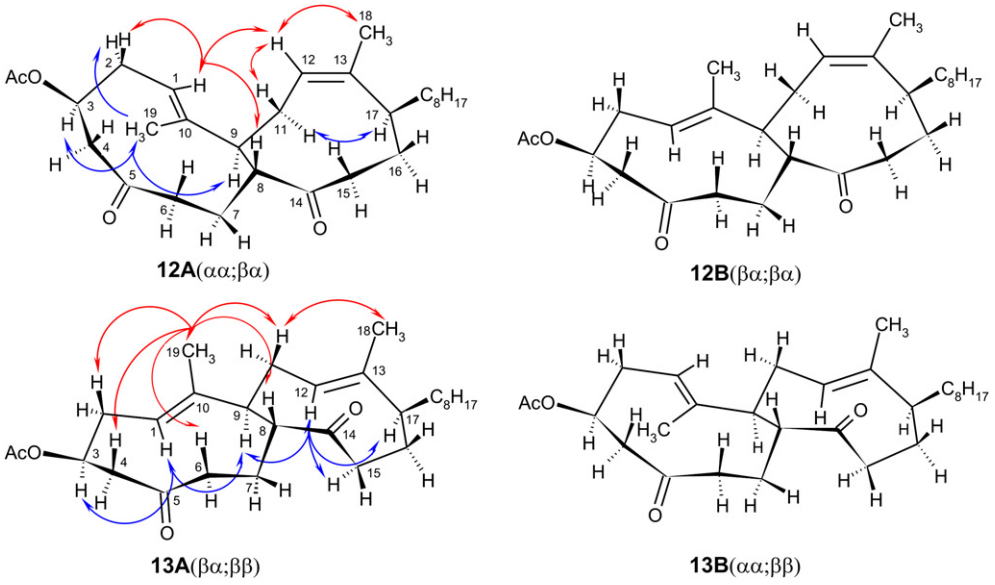


Figure 4. Solution conformations of **12** and **13** with the key NOESY correlations.

accordance to published data on solution conformations of the (*Z*)-cyclononenone in B-nor-5,10-secosteroids[6c] (Table 3, Fig. 4).

Based on the NMR data it can be concluded that the 10-membered ring conformation of **12B**[‡‡] (Table 3) matches those of conformers **6A** (Table 1) and **11A** (Table 2). The nine-membered ring conformations of **12** are similar.

According to the NMR data, (*E*,*E*)-diseco-ketone **13** exists (C_6D_6, room temperature) in two conformational forms, **A** and **B**, in the ratio of 92:8 (Table 3, Fig. 4). The distinction of the HC-1 chemical shifts of **A** and **B** conformations of **13** (δ 4.88/125.3 and 5.18/118.3 ppm, respectively), as well as the resemblance of the HC-12 chemical shifts (δ 4.84/129.9 and 4.75/129.9 ppm, respectively), suggested that the two conformations of the 10-membered ring similar to those of **6** and **11** and the same conformation of the nine-membered ring are present in solution. The presence of the NOESY H-1/H-3, H-1/H-9, and H_3-19/Hβ-2, Hβ-4, Hβ-6, H-8, as well as H-12/Hα-15, H-9, H-17 correlations, indicated that both methyl groups and C(14) carbonyl function in the main conformation **13A** are oriented toward the same side (β) of the molecule, while C(5) carbonyl function is α-oriented (Table 3, Fig. 4). Comparison of the NMR chemical shifts of HC-1 (δ 5.18/118.3 ppm), HC-3 (δ 5.28/71.7 ppm), and H_3C-19 (δ 1.60/19.8 ppm) of minor conformer **13B** (Table 3) with those of **6B** (Table 1), **11B** (Table 2), and **12A** (Table 3) suggested that the conformations of their 10-membered rings are similar (Fig. 4).

The stereoisomeric compounds **9** and **14** had the same molecular formula of $C_{29}H_{46}O_5$ established by their ESI-TOF–MS. In solution, compound **9** exists in one conformation, while **14** is present in two conformations, **A** and **B** (92:8). The NMR chemical shifts of HC-1 (δ 5.17/121.3 ppm for **9**; 4.84/125.6 ppm for **14A**), HC-3 (δ 5.61/70.8 ppm for **9**; 5.68/74.5 ppm for **14A**), HC-9 (δ 2.98/37.6 ppm for **9**; 2.23/53.0 ppm for **14A**), CH_3-19 (δ 1.34/19.42 ppm for **9**; 1.19/12.5 ppm for **14A**), as well as the presence of H-1/H-19 NOESY correlation in **9** (Table 2) and its absence in **14A** (Table 3), proved the existence of the (*Z*)- and (*E*)-1(10)-unsaturated 10-membered rings. The characteristic NMR signals of the nine-membered ring at δ 2.51/64.0, 2.31/64.8 ppm (HC-12), 62.4, 62.7 ppm (C-13), 1.04/13.8, 1.13/14.3 ppm (CH_3-18), for **9** and **14A**, respectively, revealed the presence of a 12,13-epoxide ring[5] and its stereochemistry was determined to be 12*R*,13*R* by the NOESY spectra (Tables 2 and 3, Fig. 5). The NOESY correlations of H-12 with H-9, Hα-15, and H-17, as well as the absence of H-12/CH_3-18 correlation in spectra of **9** and **14A**, proved the α-orientation of H-12 and the β-orientation of CH_3-18 group and the 14-oxo function. The NOESY connectivities on the 10-membered ring of **9** (H_3-19/H-1, H-8; Hβ-4/H-1, H-7) and **14A** (H_3-19/Hβ-2, Hβ-4, Hβ-6, H-8, Hβ-11) are in accordance with β-oriented CH_3-19 group and α-oriented 5-carbonyl group. Also, the ^{13}C NMR signals of CH_3-18 group for **9** (δ 13.8 ppm) and for **14A** (δ 14.3 ppm) are typical for its β-orientation.[4d] The upfield chemical shift of H-17 (δ 0.65 ppm for both compounds), due to the shielding effect of the epoxide function,[11] also confirms such stereochemical arrangement of the nine-membered ring in both compounds.

The ^{1}H/^{13}C chemical shifts at δ 5.07/118.5 ppm for HC-1, 5.22/71.6 ppm for HC-3, and 1.50/19.4 ppm for CH_3-19 indicated that **14B** possesses the same geometry of the 10-membered ring as it was established for **6B**, **11B**, **12A**, and **13B** (Table 3, Fig. 5). In addition, the ^{1}H/^{13}C chemical shifts at δ 1.13, 1.15/14.3 ppm for H_3C-18 in conformers **14A**,**B** suggested their nine-membered ring conformations resemblance.

The structure of compound **10** (Scheme 2) in a mixture (**9**/**10**, 75:25) has been elucidated on the basis of the molecular formula ($C_{29}H_{46}O_4$) and by the NMR data of the mixture (Table 2). The NMR chemical shifts of HC-1 (5.25dd/121.9d), HC-12 (5.31br d/127.2d), CH_3-18 (1.48s/19.4q), and CH_3-19 (1.54s/21.2q) confirm unsaturation at C-1(10) and C-12. In the COSY spectrum long-range couplings of the CH_3-19 with H-1 and the CH_3-18 with H-12 were observed. In addition, on the basis of the COSY correlations between H-1 with H_2-2, H-3 with H_2-4 together with the key HMBC correlations from H-12 to C-18, and H-9 to C-1, C-8, C-10, C-12, and C-14, the chemical shifts of the H_2C-2 (δ 2.13, 2.75/29.3 ppm), HC-3 (δ 5.71/71.3 ppm), HC-8 (δ 2.70/53.0 ppm), HC-9 (δ 3.09/39.7 ppm), C-14 (δ 213.9 ppm), HC-17 (δ 2.10/41.0 ppm) were determined. The

[‡‡] The β-oriented CH_3-19 group is too close to C(11)–C(12) bond, consequently the conformer **12B** is not the prevailing conformer.

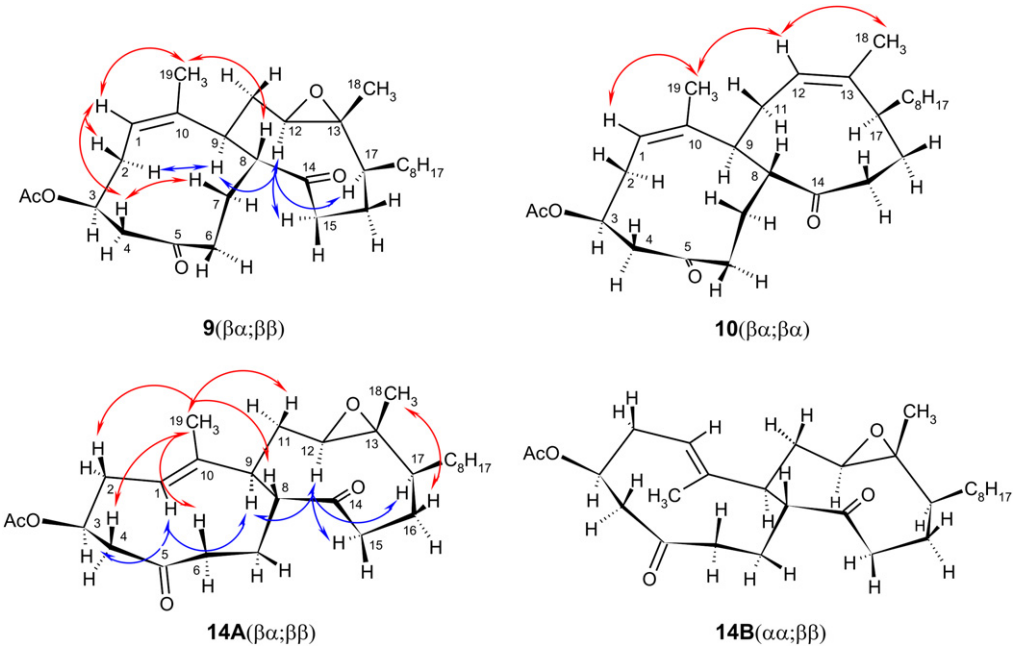


Figure 5. Solution conformations of **9**, **10**, and **14** with the key NOESY correlations.

(*Z*)-geometry of the C-1(10) and C-12 double bonds was suggested on the basis of the NOESY correlations, observed for H-1 with H_3C-19 and between H-12 and both methyl groups. The detailed comparison of the NMR data of **10** with those of (*Z*)-13,14-seco-ketone **12** and (*Z*)-5,10-seco-ketone **8**, whose stereochemistry is known, suggested that **10** might be a (*Z*,*Z*)-5,14-dioxo-5,10:13,14-disecocholesta-1(10),12-dien-3β-yl acetate in the conformation presented in Figure 5.

In solution, secosteroids **5**, **8**, **9**, and *trans*-1,10-epoxide **7** are present in a sole conformation, while secosteroids **6**, **11**, **12**, **13**, and **14** exist in two conformational forms in a different ratio (85/15–92/8), depending on the structure of C,D moiety. The conformation of the 10-membered ring of all three investigated (*Z*)-secosteroids, **5**, **8**, and **9** corresponds to that of the solid-state conformation of **5**. The conformation of (*E*)-10-membered ring (βα) for **6A**, **11A**, **12B**, **13A**, and **14A** corresponds to the solid-state conformation of **6**. In disecosteroids **9**, **13**, and **14** the olefinic CH_3-18 and 14-oxo groups are above the plane of the nine-membered ring (ββ), similarly to the nine-membered ring of B-nor-secosteroids[6c] and 13,14-secosteroids,[5] while in disecosteroid **12** the carbonyl group appears in an almost antiparallel orientation with respect to the CH_3-18 group (βα). Conformers **11B**, **12A**, **13B**, and **14B** have the same 10-membered ring conformation.

3. Conclusion

The described synthetic pathway to the novel 5,10:13,14-bis-fragmentation cholestane derivatives, which involves introduction of the 5α- and 14α-hydroxyl groups in the cholestane molecule (necessary to enable cleavage of the C(5)–C(10) and C(13)–C(14) bonds), could be used as a general method for the preparation of the same type compounds in some other steroid series. These products could be useful substrates for investigation of some interesting intramolecular processes in which the polyfunctional 10- and 9-membered rings are involved, as well as for further conformational studies. Complete and unambiguous ^1H and ^{13}C NMR resonance assignments of the obtained secosteroids, as well as the preferred conformations of their 10- and 9-membered rings were determined by extensive analysis of 2D NMR spectral data. The structures and the solid-state conformations of 5,10-secosteroids **5**–**7** were confirmed by X-ray analysis. Presented NMR data indicate that medium-sized rings in the disecosteroids studied here are not as flexible as might be expected and confirm the existence of one or two rigid conformations in C_6D_6 solution, at room temperature, depending on the ring size and the stereochemistry of the olefinic double bond. The conformational features of the 10- and 9-membered rings of the investigated secosteroids might be of interest for comparison with those of related compounds, which form the structural core of numerous bioactive natural products (i.e., germacrene sesquiterpenes compounds[12] or xenicane diterpenes[13]).

4. Experimental

4.1. General

Flash column chromatography (FCC) and TLC were carried out using Merck silica gel 0.04–0.063 mm and precoated silica gel 60 F_{254} plates, respectively. Melting points were determined on Boetius PMHK apparatus and are uncorrected. IR Spectra (ATR): Perkin–Elmer-FT-IR 1725X spectrophotometer; ν in cm^{-1}. NMR Spectra: Bruker Avance 400 MHz and 500 MHz (^1H at 400 or 500 MHz, ^{13}C at 100 or 125 MHz); $CDCl_3$ or C_6D_6 soln at room temperature; $SiMe_4$ as internal standard; δ in parts per million, J in hertz. ^1H and ^{13}C resonance assignments of all the molecules were determined with standard procedures using ^1H, ^{13}C, DEPT, COSY, HSQC, HMBC, NOESY, and TOCSY data. The temperature-dependent ^1H spectra for **5** and **6** were recorded on Bruker Avance DPX400 spectrometer in the range of 213–363 K using toulene-D_8 as solvent. Mass spectra (compounds **5**–**7**): Finnigan-MAT 8230; ionization energy 70 eV. The HRMS spectra (compounds **8**–**14**): Agilent 6210 LC ESI-MS TOF spectrometer.

4.2. Cholest-14-ene-3β,5α-diol 3-acetate (2)[7]

A gentle stream of dry HCl was passed through a solution of **1** (4.00 g, 8.99 mmol) in dry $CHCl_3$ (20 ml) at −30 °C for 3 h. The usual workup gave a residue (3.87 g), which was chromatographed on

a SiO_2 column (400 g). Elution with PhMe/Et_2O 95:5 gave 2.88 g (65%) of pure Δ^{14}-3,5-diol 3-acetate **2**, which was used in the next step without further purification: R_f=0.80 (PhMe/EtOAc 9:1); mp 144 °C (from acetone); $[\alpha]_D^{20}$ +17 (*c* 0.1, $CHCl_3$); IR: ν_{max} 3455, 1734, 1277, 1240, 1032; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.17 (s, 2H, H-3, H-15), 2.28 (ddd, 1H, *J*=2.8, 7.5, 16.0, 1H, H-16), 2.08 (m, 1H, Hβ-6), 2.08 (s, AcO), 2.02 (m, 1H, Hβ-12), 1.91 (m, 2H, Hα-2, H-16), 1.76 (m, 1H, Hα-6), 1.72 (m, 2H, H_2-4), 1.70 (m, H, Hα-7), 1.61 (m, 1H, Hα-1), 1.60 (m, 1H, Hβ-2), 1.56 (m, 1H, H-20), 1.54 (m, 1H, H-25), 1.53 (m, 1H, H-17), 1.50 (m, 1H, Hβ-1), 1.48 (m, 1H, Hα-11), 1.37 (m, 1H, H-22), 1.36 (m, 2H, Hβ-7, H-23), 1.33 (m, 1H, H-9), 1.32 (m, 2H, H-8, Hβ-11), 1.31 (m, 1H, Hα-12), 1.19 (m, 1H, H-23), 1.16 (m, 1H, H-24), 1.06 (m, 1H, H-22), 1.01 (s, 3H, CH_3-19), 0.91 (d, 3H, CH_3-21, *J*=6.8), 0.90s (CH_3-18), 0.87 (d, 6H, CH_3-26, CH_3-27, *J*=6.8); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (s, AcO), 154.9 (s, C-14), 117.2 (d, C-15), 74.7 (s, C-5), 70.8 (d, C-3), 58.7 (d, C-17), 47.1 (s, C-13), 45.4 (d, C-9), 42.2 (t, C-12), 40.0 (t, C-4), 39.5 (t, C-24), 38.9 (s, C-10), 36.0 (t, C-22), 35.6 (t, C-16), 34.1 (d, C-8), 34.0 (t, C-6), 33.9 (d, C-20), 30.5 (t, C-1), 28.0 (d, C-25), 26.7 (t, C-2), 23.8 (t, C-7), 23.7 (t, C-23), 22.8, 22.5 (two q, C-26 and C-27), 22.0 (t, C-11), 21.4 (q, AcO), 18.9 (q, C-21), 16.9 (q, C-18), 15.8 (q, C-19); 2D NOESY correlations H-3/Hα-4, Hα-2, Hα-1; H-15/Hα,β-16; CH_3-18/H-8, Hβ-11; CH_3-19/H-8, Hβ-2, Hβ-4, Hβ-6, Hβ-11; ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{48}O_3Na$: 467.34957 $[M+Na]^+$, found 467.34620.

4.3. 14α,15-Epoxycholestane-3β,5α-diol 3-acetate (3)[7]

To a stirred solution of **2** (2.80 g, 6.30 mmol) in CH_2Cl_2 (50 ml) *m*-chloroperbenzoic acid (1.52 g, 8.82 mmol) was added, and the mixture left at room temperature for 1 h. The reaction mixture was worked up as usually. The crude product was crystallized from acetone/MeOH to give 2.67 g (92%) of 14α,15α-epoxide **3**: R_f=0.20 (PhMe/EtOAc 9:1); mp 146–147 °C (from MeOH/acetone); $[\alpha]_D^{20}$ +10 (*c* 0.1, $CHCl_3$); IR: ν_{max} 3507, 1737, 1268, 1251, 1030; ^{1}H NMR (500 MHz, C_6D_6): δ 5.51 (hept., *J*=6.0, 1H, H-3), 3.19 (s, 1H, H-15), 2.11 (dd, *J*=6.5, 13.0, 1H, Hα-16), 1.98 (m, 3H, Hα-2, H-8, H-9), 1.82 (m, 1H, Hβ-12), 1.79 (m, 1H, Hα-1), 1.78 (s, 3H, AcO), 1.78 (m, 1H, Hα-4), 1.76 (m, 1H, Hα-7), 1.66 (m, 1H, Hα-12), 1.63 (m, 1H, Hβ-4), 1.58 (m, 1H, Hβ-2), 1.53 (m, 1H, H-25), 1.44 (m, 1H, Hβ-6), 1.39 (m, 1H, H-17), 1.38 (m, 1H, Hα-11), 1.37 (m, 1H, H-23), 1.36 (m, 1H, H-22), 1.33 (m, 1H, H-20), 1.29 (m, 1H, Hβ-1), 1.20 (m, 1H, Hβ-11), 1.17 (m, 2H, H-23, H-24), 1.10 (m, 1H, Hα-6), 1.03 (dd, *J*=10.0, 13.0, 1H, Hβ-16), 1.02 (m, 1H, H-22), 0.919, 0.922 (2d, *J*=6.5, 6H, CH_3-26, CH_3-27), 0.89 (m, 1H, Hβ-7), 0.89 (d, *J*=6.5, 3H, CH_3-21), 0.82 (s, 3H, CH_3-19), 0.74 (s, 3H, CH_3-18); ^{13}C NMR (125 MHz, C_6D_6): δ 170.3 (s, AcO), 74.3 (s, C-5), 73.9 (s, C-14), 71.4 (d, C-3), 58.0 (d, C-15), 49.4 (d, C-17), 42.7 (d, C-9), 41.8 (s, C-13), 40.8 (t, C-4), 40.3 (t, C-24), 39.4 (s, C-10), 36.6 (t, C-22), 36.5 (t, C-12), 34.6 (d, C-20), 34.1 (t, C-6), 32.8 (t, C-16), 32.0 (d, C-8), 31.2 (t, C-1), 28.7 (d, C-25), 27.8 (t, C-2), 24.7 (t, C-23), 23.4, 23.1 (two q, C-27 and C-26), 21.8 (t, C-11), 21.5 (q, AcO), 20.3 (t, C-7), 19.4 (q, C-21), 16.2 (q, C-19), 15.1 (q, C-18); 2D NOESY correlations H-3/Hα-4, Hα-2; H-15/Hα,β-7, Hα,β-16; H-8/Hβ-7; CH_3-18/H-8, Hβ-12, H-20, Hβ-11, Hβ-16; CH_3-19/H-8, Hβ-2, Hβ-4, Hβ-6, Hβ-1, Hβ-11; H17/Hα-16; ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{48}O_4K$: 499.31842 $[M+K]^+$, found 499.31628.

4.4. Cholestane-3β,5α,14α-triol 3-acetate (4)[7]

The epoxide **3** (2.50 g, 5.43 mmol) was reduced with $LiAlH_4$ (0.51 g, 13.44 mmol) in dry Et_2O (50 ml) at reflux for 48 h. The reaction mixture was worked up in the usual manner to give 3β,5α,14α-triol (2.26 g), which was acetylated with Ac_2O (25 ml) in anhyd pyridine (20 ml) overnight at room temperature. Usual workup gave a residue, which was crystallized from acetone to give 1.63 g (65%) 5,14-diol **4**: R_f=0.17 (PhMe/EtOAc 8:2); mp 194–195 °C (from acetone); $[\alpha]_D^{20}$ +23.64 (*c* 0.1269, $CHCl_3$); IR: ν_{max} 3495, 3445, 1737, 1274, 1254, 1031; ^{1}H NMR (500 MHz, C_6D_6): δ 5.46 (hept., *J*=6.0, H-3), 3.09 (s, C(5)–OH), 2.48 (s, C(14)–OH), 2.30 (m, 1H, Hα-16), 2.14 (m, 1H, H-17), 2.11 (m, 1H, H-9), 2.06 (m, 1H, Hα-7), 2.05 (m, 1H, Hα-12), 1.94 (m, 1H, Hα-2), 1.90 (m, 1H, Hα-4), 1.82 (s, AcO), 1.82 (m, 1H, Hα-1), 1.70 (m, 1H, Hβ-12), 1.68 (m, 2H, H-8, Hβ-4), 1.64 (m, 1H, H-22), 1.62 (m, 1H, H-25), 1.60 (m, 1H, Hβ-2), 1.58 (m, 1H, Hα-15), 1.57 (m, 1H, H-23), 1.53 (m, 1H, Hβ-6), 1.49 (m, 1H, H-20), 1.42 (m, 1H, Hβ-15), 1.34 (m, 3H, Hα-1, Hα-11, Hβ-16), 1.33 (m, 1H, Hα-6), 1.31 (m, 2H, H-23, H-24), 1.25 (m, 1H, Hβ-11), 1.22 (m, 1H, H-22), 1.08 (d, *J*=6.0, 3H, CH_3-21), 0.98, 0.99 (2d, *J*=6.5, 6H, CH_3-26, CH_3-27), 0.83 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18); ^{13}C NMR (125 MHz, C_6D_6): δ 170.8 (s, AcO), 86.0 (s, C-14), 75.6 (s, C-5), 71.4 (d, C-3), 51.9 (d, C-17), 47.5 (s, C-13), 40.8 (t, C-4), 40.4 (t, C-24), 39.4 (s, C-10), 38.7 (d, C-9), 38.5 (d, C-8), 37.5 (t, C-22), 36.9 (d, C-20), 34.9 (t, C-6), 33.8 (t, C-15), 33.3 (t, C-12), 31.9 (t, C-1), 28.8 (d, C-25), 28.3 (t, C-16), 27.9 (d, C-2), 25.4 (t, C-23), 23.5, 23.2 (two q, C-27 and C-26), 21.5 (q, AcO), 21.4 (t, C-7), 21.3 (t, C-11), 19.4 (q, C-21), 16.6 (q, C-18), 16.3 (q, C-19); 2D NOESY correlations H-3/Hα-4, Hα-2, C(5)–OH; H-9/C(5)–OH, C(14)–OH; CH_3-18/H-8, H-20, Hβ-11, Hβ-15, CH_3-21; CH_3-19/H-8, Hβ-2, Hβ-4, Hβ-6, Hβ-1, Hβ-11; CH_3-21/H-20, Hβ-12; ESI-TOF–MS: *m*/*z*: calcd for $C_{30}H_{51}O_6$: 507.36911 $[M+HCOO]^-$, found 507.36765.

4.5. Hypoiodite HgO oxidation of 4

A stirred suspension of 5α,14α-diol **4** (500 mg, 1.08 mmol), yellow HgO (1.400 g, 6.48 mmol), and I_2 (2.822 g, 11.12 mmol) in dry CCl_4 (350 ml) was irradiated with an 800 W OSRAM Halogen-Bellaphot lamp (at 100 V) at room temperature for 4 h. The mixture was filtered, the filtrate washed successively with aq $Na_2S_2O_3$ soln, aq $NaHCO_3$ soln, and H_2O, dried (Na_2SO_4), evaporated, and the resulting mixture separated by FCC, PhMe/EtOAc (0→20).

Elution with PhMe/EtOAc 95:5 gave the (*Z*)-5-oxo-5,10-secocholest-1(10)-ene-3β,14α-diol 3-acetate (**5**) (49.8 mg, 10%): R_f=0.64 (PhMe/EtOAc 8:2); mp 162–163 °C (from MeOH/acetone); $[\alpha]_D^{20}$ +61.9 (*c* 0.1163, $CHCl_3$); IR: ν_{max} 3577, 3510, 1732, 1699, 1656, 1466, 1441, 1376, 1240, 1031; ^{1}H and ^{13}C NMR (400 and 100 MHz, C_6D_6) see Table 1; MS: 460 (M^+), 400 (M^+−60), 382 (M^+−60–18).

Elution with PhMe/EtOAc 90:10 gave the (*E*)-5-oxo-5,10-secocholest-1(10)-ene-3β,14α-diol 3-acetate (**6**) (264 mg, 53%): R_f=0.49 (PhMe/EtOAc 8:2); mp 127–128 °C (from MeOH/acetone); $[\alpha]_D^{20}$ +27.3 (*c* 0.1247, $CHCl_3$); IR: ν_{max} 3581, 3520, 1725, 1699, 1656, 1464, 1368, 1256, 1034; ^{1}H and ^{13}C NMR (400 and 100 MHz, C_6D_6) see Table 1; MS: 442 (M^+−18), 400 (M^+−60).

Further elution with PhMe/EtOAc 88:12 afforded 1*R*,10*R*-epoxy-5-oxo-5,10-secocholestane-3β,14α-diol 3-acetate (**7**) (31 mg, 6.0%): R_f=0.40 (PhMe/EtOAc 8:2); mp 181–182 °C (from MeOH/acetone); $[\alpha]_D^{20}$ +32.0 (*c* 0.1156, $CHCl_3$); IR: ν_{max} 3521, 1726, 1462, 1380, 1261, 1127, 1031; ^{1}H and ^{13}C NMR (400 and 100 MHz, $CDCl_3$) see Table 1; MS: 476 (M^+), 458 (M^+−18), 416 (M^+−60), 398 (M^+−60–18).

*4.5.1. X-ray analysis of **5**–**7**.* The compounds were recrystallized by slow evaporation from a mixture acetone/ethanol/hexane for **5**; from ethanol for **6** and from acetone for **7**.

The X-ray intensity data were collected at 120 K for the three compounds with an MAR345 image plate using Mo Kα (λ=0.71069Å) radiation. The crystal was chosen, mounted in inert oil, and transferred to the cold gas stream for flash cooling. The crystal data and the data collection parameters are summarized in Table 4. The unit cell parameters were refined using all the collected spots after the integration process. The data were not corrected for absorption, but the data collection mode with high redundancy, partially takes the absorption phenomena into account.

The structures were solved by direct methods and refined by full-matrix least-squares on F^2 using SHELXL97.[14] All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms

Table 4
Crystal data and structure refinement for 5,10-secosteroids **5**–**7**

	5	**6**	**7**
Empirical formula	$C_{29}H_{48}O_4$	$C_{29}H_{48}O_4$	$C_{29}H_{48}O_5$
Formula weight	460.69	460.69	476.69
Temperature	120(2) K	120(2) K	120(2) K
Wavelength	0.71069 Å	0.71069 Å	0.71069 Å
Crystal system, space group	Monoclinic, *P*21	Monoclinic, *C*2	Monoclinic, *P* 21
Unit cell dimensions	a=20.720(5) Å α=90°	a=19.990(7) Å α=90°	a=7.414(2) Å α=90°
	b=5.7630(10) Å β=92.38°	b=9.173(3) Å β=111.03°	b=35.701(9) Å β=109.78°
	c=22.700(5) Å γ=90°	c=15.883(5) Å γ=90°	c=10.882(3) Å γ=90°
Volume	2708.3(10) Å^3	2718.4(16) Å^3	2710.4(12) Å^3
Z, calculated density	2; 1.130 Mg/m^3	4; 1.126 Mg/m^3	4; 1.168 Mg/m^3
Absorption coefficient	0.073 mm^{-1}	0.073 mm^{-1}	0.078 mm^{-1}
F(000)	1016	1016	1048
Crystal size	0.7×0.1×0.08 mm (needle)	0.35×0.30×0.08 mm	0.55×0.30×0.05 mm
Θ Range for data collection	2.95–20.39°	3.78–23.52°	3.42–22.23°
Limiting indices	$-19\le h\le 20$; $-5\le k\le 5$; $-22\le l\le 22$	$-22\le h\le 22$; $-10\le k\le 10$; $-17\le l\le 17$	$-7\le h\le 7$; $-37\le k\le 37$; $-11\le l\le 11$
Reflections collected, unique	10,468, 4899 [R(int)=0.095]	17,349, 4018 [R(int)=0.049]	12,161, 6169 [R(int)=0.035]
Completeness to	Θ=20.39 (96.6%)	Θ=23.52 (99.3%)	Θ=22.23 (96.2%)
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data, restraints, parameters	4899, 4, 596	4018, 1, 301	6169, 13, 609
Goodness-of-fit on F^2	1.059	1.127	1.108
Final R indices [$I>2\sigma(I)$]	R_1=0.0824, [4372], wR_2=0.2060	R_1=0.0438, [3933] wR_2=0.1193	R_1=0.1261, [5175] wR_2=0.3195
R indices (all data)	R_1=0.0922, wR_2=0.2161	R_1=0.0446, wR_2=0.1201	R_1=0.1276, wR_2=0.3204
Absolute structure parameter	0(3)	0.4(12) not reliable	0(4) not reliable
Extinction coefficient	0.036(3)	—	0.023(3)
Largest diff. peak and hole	0.490 and −0.352 e Å^{-3}	0.290 and −0.299 e Å^{-3}	0.484 and −0.447 e Å^{-3}

were calculated with AFIX and included in the refinement with a common isotropic temperature factor. The details of the refinement and the final R indices are presented in Table 4. The R indices for **7** are high because of poor crystal quality but different trials to obtain better crystals were unsuccessful.

Further details of crystal structure have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers: CCDC 725173 (**5**); 725174 (**6**), and 725175 (**7**)). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.6. Hypoiodite LTA oxidation of seco-ketones 5 and 6

4.6.1. General procedure. A mixture of seco-ketone (200 mg, 0.43 mmol), LTA (1.200 g, 2.706 mmol), and I_2 (240 mg, 0.94 mmol) in CCl_4 (40 ml) was stirred and irradiated with a 800 W OSRAM Halogen-Bellaphot lamp (at 140 V) at room temperature for 5 h (for **5**) and 2 h (for **6**). The reaction mixture was filtered off and the filtrate washed successively with aq $NaHCO_3$ soln, and H_2O, dried (Na_2SO_4), and evaporated. The residue was chromatographed by FCC using below mentioned solvents as eluents.

*4.6.2. Hypoiodite LTA oxidation of **5**.* Elution with hexane/EtOAc 85:15 (→**8**), 75/25→(**9**+**10**): **8** (15.2 mg, 8%), **9**+**10** (82.6 mg, ~40%). (*Z*)-13*R*,14-Epoxy-5-oxo-5,10:13,14-disecocholesta-1(10),14-dien-3β-yl acetate (**8**): R_f=0.60 (hexane/EtOAc 7:3); oil; $[\alpha]_D^{20}$ +145.4 (*c* 0.11, $CHCl_3$); IR: ν_{max} 2950, 2921, 2866, 1737, 1704, 1673, 1439, 1369, 1231, 1019; ^{1}H and ^{13}C NMR (500 and 125 MHz, C_6D_6) see Table 2; ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{47}O_4$: 459.34689 $[M+H]^+$, found 459.34588.

The inseparable mixture of (*Z*)-12*R*,13*R*-epoxy-5,14-dioxo-5,10:13,14-disecocholest-1(10)-en-3β-yl acetate (**9**) and (*Z*,*Z*)-5,14-dioxo-5,10:13,14-disecocholesta-1(10),12-dien-3β-yl acetate (**10**) (~40%, 75:25): R_f=0.35 (hexane/EtOAc 7:3); oil; IR: ν_{max} 2953, 2928, 2867, 1736, 1704, 1688, 1441, 1366, 1233, 1026; ^{1}H and ^{13}C NMR (500 and 125 MHz, C_6D_6) see Table 2. ESI-TOF–MS: **9**: *m*/*z*: calcd for $C_{29}H_{47}O_5$: 475.34180 $[M+H]^+$, found 475.34202; *Z*,*Z*-di-seco-ketone **10**: *m*/*z*: calcd for $C_{29}H_{46}O_4Na$: 481.32883 $[M+Na]^+$, found 481.32836.

*4.6.3. Hypoiodite LTA oxidation of **6**.* Elution with hexane/Et_2O/EtOAc 70:15:15 (→ **11**, **12**, **13**), 60:20:20→(**14**): **11** (10 mg, 5%), **12** (41.8 mg, 21%), **13** (59.7 mg, 30%), **14** (18.5 mg, 9%).

(*E*)-13*R*,14-Epoxy-5-oxo-5,10:13,14-disecocholesta-1(10),14-dien-3β-yl acetate (**11**): R_f=0.59 (hexane/EtOAc 7:3); mp 131–134 °C; $[\alpha]_D^{20}$ +53.3 (*c* 0.12, $CHCl_3$); IR: ν_{max} 2951, 2926, 2867, 1736, 1703, 1678, 1365, 1232, 1023; ^{1}H and ^{13}C NMR (500 and 125 MHz, C_6D_6) see Table 2. ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{46}O_4K$: 497.30277 $[M+K]^+$, found 497.30326.

(*E*,*Z*)-5,14-Dioxo-5,10:13,14-disecocholesta-1(10),12-dien-3β-yl acetate (**12**): R_f=0.48 (hexane/EtOAc 7:3); mp 155–157 °C; $[\alpha]_D^{20}$ +63.6 (*c* 0.11, $CHCl_3$); IR: ν_{max} 2954, 2928, 2868, 1738, 1702, 1447, 1235, 1028; ^{1}H and ^{13}C NMR (500 and 125 MHz, C_6D_6) see Table 3. ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{46}O_4K$: 497.30277 $[M+K]^+$, found 497.30164.

(*E*,*E*)-5,14-Dioxo-5,10:13,14-disecocholesta-1(10),12-dien-3β-yl acetate (**13**): R_f=0.41 (hexane/EtOAc 7:3); mp 134–135 °C; $[\alpha]_D^{20}$ −3.6 (*c* 0.11, $CHCl_3$); IR: ν_{max}=2953, 2925, 2868, 1735, 1699, 1681, 1446, 1365, 1237, 1032; ^{1}H and ^{13}C NMR (500 and 125 MHz, C_6D_6) see Table 3. ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{46}O_4K$: 497.30277 $[M+K]^+$, found 497.30104.

(*E*)-12*R*,13*R*-Epoxy-5,14-dioxo-5,10:13,14-disecocholest-1(10)-en-3β-yl acetate (**14**): R_f=0.24 (hexane/EtOAc 7:3); mp 177–179 °C; $[\alpha]_D^{20}$ −10.0 (*c* 0.13, $CHCl_3$); IR: ν_{max} 2952, 2929, 2868, 1738, 1698, 1446, 1362, 1236, 1030; ^{1}H and ^{13}C NMR (500 and 125 MHz, C_6D_6) see Table 3. ESI-TOF–MS: *m*/*z*: calcd for $C_{29}H_{47}O_5$: 475.34180 $[M+H]^+$, found 475.34049.

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