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Catalytic cyclometallation in steroid chemistry III[1]: Synthesis of steroidal derivatives of 5Z,9Z-dienoic acid and investigation of its human topoisomerase I inhibitory activity

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ABSTRACT

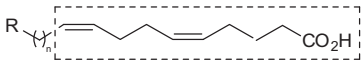
Two approaches to stereoselective synthesis of steroid 5Z,9Z-dienoic acids were developed, the first one being based on the cross-cyclomagnesiation of 2-(hepta-5,6-dien-1-yloxy)tetrahydro-2H-pyran and 1,2-diene cholesterol derivatives on treatment with EtMgBr catalyzed by Cp_2TiCl_2, while the other involving the synthesis of esters of hydroxy steroids with (5Z,9Z)-tetradeca-5,9-dienedioic acid, prepared in two steps using homo-cyclomagnesiation of 2-(hepta-5,6-dien-1-yloxy)tetrahydro-2H-pyran as the key step. High inhibitory activity of the synthesized acids against human topoisomerase I (hTop1) was found.

1. Introduction

In recent years, considerable attention has been paid to the search for new inhibitors of enzymes that synthesize or modify nucleic acids. An important enzyme in this series is DNA-dependent topoisomerase I, which catalyzes the topological rearrangements of DNA and plays key role in all aspects of genome functioning [1–3]. The topoisomerase-induced breaks in one (topoisomerase I) or two (topoisomerase II) DNA strands followed by repair and restoration of integrity of the DNA molecule provides the mobility needed for conformational changes of DNA in the template-directed synthesis and chromosome mobility during mitosis. Topoisomerases are considered as intracellular targets for chemotherapeutic agents, as by preventing the break repairs, these substances can induce accumulation of damaged DNA molecules, thus promoting the cell death [1–3].

Extensive data on the synthesis of topoisomerase inhibitors have been reported in the literature. They were found among compounds of various classes, which can be used to elucidate the structure–property relationships in order to optimize the known pharmaceutical drugs and synthesize new ones [2,3].

Natural 5Z,9Z-dienoic acids isolated from sea sponges and fruits of gymnosperms exhibit high inhibitory activity towards human topoisomerase I [4–7]. As a development of these studies, by considering carboxylic acids of various structures, it was found that the presence of 1-carboxy-5Z,9Z-diene group in the structure of molecule is obviously correlated with the inhibitory activity towards topoisomerase I and IIα exhibited by this acid [8]. The nature of the substituent at the 1-carboxy-5Z,9Z-diene group can, in turn, enhance or mitigate its action and endow the molecule with additional properties (lipophilicity, transport function, solubility, etc.) [9].



Note that synthetic routes to 5Z,9Z-dienoic acids reported in the literature comprise multiple steps (5–20) and give target compounds

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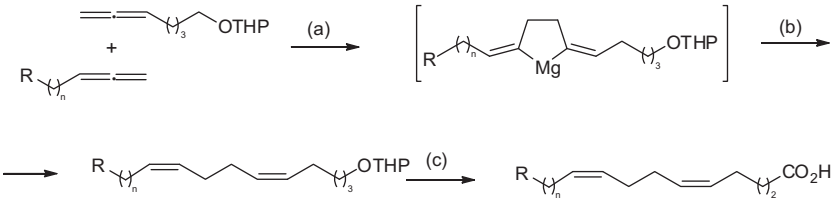


Scheme 1. Method for the synthesis of 5Z,9Z-dienoic acids. (a): EtMgBr, Mg, Cp_2TiCl_2 (10 mol%), diethyl ether; (b): H_3O^+; (c): H_2CrO_4/H_2SO_4, acetone, CH_2Cl_2.

in only 0.5–15% yields; moreover, in most cases, the reactions result in mixtures of stereoisomers, which seems to be the key issue that hampers further investigation and application of this class of compounds to design modern pharmaceutical drugs [6,10].

An effective method for the formation of 1-carboxy-5Z,9Z-diene moiety is, in our opinion, the method based on the use of the new reaction of Ti-catalyzed cross-cyclomagnesiation of O-containing and aliphatic 1,2-dienes on treatment with Grignard reagents (Scheme 1) [11–13].

In order to determine the scope of applicability of the cross-cyclomagnesiation of 1,2-dienes we developed original methods and approaches to the synthesis of new derivatives of 5Z,9Z-dienoic acids containing a steroid core, and elucidated the effect of the structure of the substituent at the 1-carboxy-5Z,9Z-diene moiety on the inhibitory activity against human topoisomerase I. Here we present the results concerning cross-cyclomagnesiation of cholesterol derivatives of 1,2-dienes with the tetrahydropyran ether of hepta-5,6-dien-1-ol and the synthesis of esters of hydroxy steroids with (5Z,9Z)-tetradeca-5,9-dienedioic acids.

2. Experimental

All solvents were dried (1,4-dioxane, diethyl ether over Na) and freshly distilled before use. All reactions were carried out under a dry argon atmosphere. ^1H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 500 spectrometer in $CDCl_3$ operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Melting points were recorded on Stuart SMP3. Mass spectra were obtained on MALDI TOF/TOF spectrometer in a α-cyano-4-hydroxycinnamic acid matrix. Elemental analyses were measured on a 1106 Carlo Erba apparatus. Individuality and purity of the synthesized compounds were controlled using TLC on Sorbfil plates; anisic aldehyde in acetic acid was used as a developer. Column chromatography was carried out on Acrus silica gel (0.060–0.200 mm). The THP ether of 5,6-hepta-5,6-dien-1-ol **4**, was prepared from commercially available hex-5-yn-1-ol by a reported procedure [12].

*2.1. Ether-derivatives cholesterol (**2a**) and (**2b**) prepared by a reported procedure [14]*

*2.1.1. (3β)-3-(hex-5-yn-1-yloxy)cholest-5-ene (**2b**)*

Compound (**2b**) was purified by column chromatography (hexane/ethyl acetate = 30/1) as a white crystals. Yield: 71%; m.p. 45–47 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.35 (m, 1H, H-6), 3.49 (t, *J* = 6.0 Hz, 2H, CH_2O), 3.13 (m, 1H, H-3), 2.23 (m, 2H, $CHCCH_2$), 1.95 (t, *J* = 2.5 Hz, 1H, $CHCCH_2$), 1.68 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 1.01 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 141.0 (C), 121.5 (CH), 84.4 (C), 79.0 (CH), 68.4 (CH), 67.3 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 31.9 (CH_2), 31.9 (CH), 29.3 (CH_2), 28.5 (CH_2), 28.3 (CH_2), 28.0 (CH), 25.3 (CH_2), 24.3 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.8 (CH_3), 18.3 (CH_2), 11.9 (CH_3).

*2.2. Synthesis of 1,2-dienes derivatives of cholesterol **3a** and **3b***

Paraformaldehyde (79 mg), copper iodide (21 mg, 0.1 mmol), and diisopropylamine (0.28 ml, 2 mmol) were sequentially added to a solution of compound (**2a**) or (**2b**) (1.0 mmol) in anhydrous dioxane (15 ml). The resulting mixture was refluxed for 24 h. The addition of 2 M HCl (10 ml) and extraction with diethyl ether was followed by an extraction of the organic layer with $NaHCO_3$, water, and brine and a drying with anhydrous $MgSO_4$. The solvent was evaporated in vacuo, and the residue was purified by column chromatography using hexane/ethyl acetate = 30/1 as the elution solvent to afford cholesterol derivatives of 1,2-diene (**3a**) or (**3b**).

*2.2.1. (3β)-3-(penta-3,4-dien-1-yloxy)cholest-5-ene (**3a**)*

White crystals. Yield: 0.22 g (50%); m.p. 42–44 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.36 (m, 1H, H-6), 5.16 (m, 1H, CH_2CCH), 4.69 (m, 2H, CH_2CCH), 3.56 (t, *J* = 6.5 Hz, 2H, CH_2O), 3.18 (m, 1H, H-3), 2.29 (m, 2H, $CHCCH_2CH_2$), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.89 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 208.9 (C), 141.1 (C), 121.5 (CH), 86.8 (CH), 79.0 (CH), 74.9 (CH_2), 67.3 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 31.9 (CH_2), 31.9 (CH), 29.2 (CH_2), 28.5 (CH_2), 28.3 (CH_2), 28.0 (CH), 24.3 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3).

*2.2.2. (3β)-3-(hepta-5,6-dien-1-yloxy)cholest-5-ene (**3b**)*

White crystals. Yield: 0.23 g (48%); m.p. 62–64 °C. ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.35 (m, 1H, H-6), 5.11 (m, 1H, CH_2CCH), 4.67 (m, 2H, CH_2CCH), 3.48 (t, *J* = 6.5 Hz, 2H, CH_2O), 3.14 (m, 1H, H-3), 2.05 (m, 2H, $CHCCH_2CH_2$), 1.62 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 208.6 (C), 141.1 (C), 121.4 (CH), 89.9 (CH), 78.9 (CH), 74.7 (CH_2), 67.8 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 31.9 (CH_2), 31.9 (CH), 29.6 (CH_2), 28.5 (CH_2), 28.3 (CH_2), 28.1 (CH_2), 28.0 (CH), 25.8 (CH_2), 24.3 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3).

*2.3. Cross-cyclomagnesiation of 1,2-dienes derivatives of cholesterol **3a** and **3b** with THP ether of 5,6-hepta-5,6-dien-1-ol **4** by EtMgBr in the presence of Mg metal and Cp_2TiCl_2 catalyst (general procedure)*

Diethyl ether (10 ml), allenic esters of cholesterol (**3a**) or (**3b**) (1.0 mmol), THP ether of 5,6-hepta-5,6-dien-1-ol (**4**) (0.59 g, 3.0 mmol), EtMgBr (5.3 ml, 8.0 mmol) (as 1.5 M solution in Et_2O), Mg powder (0.29 g, 12.0 mmol) and Cp_2TiCl_2 (24.9 mg, 0.1 mmol) were charged into a glass reactor with stirring under argon

(~0 °C). The reaction mixture was warmed-up to room temperature (20–22 °C) and stirred for 24 h. The reaction mixture was treated with a 5% solution of NH_4Cl in H_2O (20 ml). The products were extracted with diethyl ether (2 × 50 ml), the extracts were dried with $MgSO_4$, the solvent was evaporated, and the residue was chromatographed on a column (SiO_2, elution with petroleum ether/EtOAc (35/1)).

*2.3.1. (3β)-3-{[(3Z,7Z)-12-(tetrahydro-2H-pyran-2-yloxy)dodeca-3,7-dien-1-yl]oxy}cholest-5-ene (**6a**)*

Colorless waxy solid. Yield: 0.23 g (35%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.46 (m, 1H, HC=), 5.43 (m, 1H, HC=), 5.40 (m, 2H, HC=), 5.35 (m, 1H, H-6), 4.59 (m, 1H, CH_2CHO), 3.88 and 3.51 (m, 2H, CH_2O), 3.76 and 3.41 (m, 2H, CH_2O), 3.48 (m, 2H, CH_2O), 3.16 (m, 1H, H-3), 2.33 (m, 2H, CH_2CH=), 2.11 (m, 4H, CH_2CH=), 2.08 (m, 2H, CH_2CH=), 1.85 and 1.53 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 141.1 (C), 131.2 (CH), 130.1 (CH), 129.3 (CH), 126.02 (CH), 121.5 (CH), 98.8 (CH), 79.0 (CH), 67.6 (CH_2), 67.5 (CH_2), 62.3 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 31.9 (CH_2), 31.9 (CH), 30.8 (CH_2), 29.4 (CH_2), 28.5 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 28.0 (CH), 27.5 (CH_2), 27.3 (CH_2), 27.1 (CH_2), 26.4 (CH_2), 25.5 (CH_2), 24.3 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.7 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3).

*2.3.2. (3β)-3-{[(5Z,9Z)-14-(tetrahydro-2H-pyran-2-yloxy)tetradeca-5,9-dien-1-yl]oxy}cholest-5-ene (**6b**)*

Colorless waxy solid. Yield: 0.28 g (42%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.39 (m, 4H, HC=), 5.35 (m, 1H, H-6), 4.59 (m, 1H, CH_2CHO), 3.89 and 3.53 (m, 2H, CH_2O), 3.76 and 3.40 (m, 2H, CH_2O), 3.46 (m, 2H, CH_2O), 3.15 (m, 1H, H-3), 2.10 (m, 4H, CH_2CH=), 2.08 (m, 4H, CH_2CH=), 1.85 and 1.54 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 1.44 (m, 4H, CH_2), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 141.2 (C), 130.0 (CH), 129.9 (CH), 129.5 (CH), 129.4 (CH), 121.5 (CH), 98.8 (CH), 78.9 (CH), 67.9 (CH_2), 67.5 (CH_2), 62.3 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.2 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 31.9 (CH_2), 31.9 (CH), 30.8 (CH_2), 29.8 (CH_2), 29.4 (CH_2), 28.5 (CH_2), 28.2 (CH_2), 28.0 (CH), 27.4 (CH_2), 27.4 (CH_2), 27.1 (CH_2), 27.1 (CH_2), 26.4 (CH_2), 26.4 (CH_2), 25.5 (CH_2), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.7 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3).

*2.4. Oxidation of compound **6a** and **6b** with Jones reagent*

To a solution of compound **6a** or **6b** (0.5 mmol) in acetone (12 ml) and CH_2Cl_2 (2 ml) at rt, Jones reagent (0.5 ml) was added dropwise. The reaction mixture was stirred at rt for 1 h, then quenched with water (5 ml), concentrated under reduced pressure to remove the excess of acetone and CH_2Cl_2, and the aqueous layer extracted with diethyl ether (3 × 10 ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography using hexane/ethyl acetate = 5/1 as the elution solvent to afford 5Z,9Z-dienoic acids **7a** or **7b**.

*2.4.1. (5Z,9Z)-12-[(3β)-cholest-5-en-3-yloxy]dodeca-5,9-dienoic acid (**7a**)*

White waxy solid. Yield 0.15 g (51%), $[\alpha]_D^{20}$ – 13.8 (*c* 1.09, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.47 (m, 2H, HC=), 5.40 (m, 1H, HC=), 5.36 (m, 1H, H-6), 5.35 (m, 1H, HC=), 3.49 (t, *J* = 7.5 Hz, 2H, CH_2O), 3.21 (m, 1H, H-3), 2.37 (m, 2H, CH_2COOH), 2.35 (m, 2H, CH_2CH=), 2.14 (m, 2H, CH_2CH=), 2.11 (m, 4H, CH_2CH=), 1.72 (m, 2H, CH_2), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 178.7 (C), 140.9 (C), 131.1 (CH), 130.4 (CH), 128.8 (CH), 126.0 (CH), 121.6 (CH), 79.2 (CH), 67.7 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.1 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 33.2 (CH_2), 31.9 (CH_2), 31.9 (CH), 28.4 (CH_2), 28.4 (CH_2), 28.3 (CH_2), 28.0 (CH), 27.5 (CH_2), 27.4 (CH_2), 26.4 (CH_2), 24.6 (CH_2), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3). MALDI TOF: *m*/*z* 603.039 ($[M+Na]^+$, calcd 603.475). Anal. Calcd for $C_{39}H_{64}O_3$: C, 80.63; H, 11.10. Found C, 80.49; H, 11.07.

*2.4.2. (5Z,9Z)-14-[(3β)-cholest-5-en-3-yloxy]tetradeca-5,9-dienoic acid (**7b**)*

White waxy solid. Yield 0.16 g (53%), $[\alpha]_D^{20}$ – 19.6 (*c* 1.39, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.43 (m, 2H, HC=), 5.38 (m, 2H, HC=), 5.35 (m, 1H, H-6), 5.33 (m, 1H, HC=), 3.49 (m, 2H, CH_2O), 3.16 (m, 1H, H-3), 2.36 (m, 2H, CH_2COOH), 2.13 (m, 4H, CH_2CH=), 2.13 (m, 2H, CH_2CH=), 2.09 (m, 4H, CH_2CH=), 1.71 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 1.43 (m, 2H, CH_2), 1.02 (s, 3H, H-19), 0.93 (d, *J* = 6.5 Hz, 3H, H-21), 0.88 (d, *J* = 6.5 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 179.0 (C), 141.0 (C), 130.5 (CH), 130.1 (CH), 129.3 (CH), 128.8 (CH), 121.5 (CH), 79.2 (CH), 68.0 (CH_2), 56.8 (CH), 56.2 (CH), 50.2 (CH), 42.3 (C), 39.8 (CH_2), 39.5 (CH_2), 39.1 (CH_2), 37.3 (CH_2), 36.9 (C), 36.2 (CH_2), 35.8 (CH), 33.3 (CH_2), 31.9 (CH_2), 31.9 (CH), 29.7 (CH_2), 28.4 (CH_2), 28.3 (CH_2), 28.0 (CH), 27.5 (CH_2), 27.3 (CH_2), 27.1 (CH_2), 26.5 (CH_2), 26.3 (CH_2), 24.6 (CH_2), 24.3 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.1 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3). MALDI TOF: *m*/*z* 631.244 ($[M+Na]^+$, calcd 631.507). Anal. Calcd for $C_{41}H_{68}O_3$: C, 80.86; H, 11.25. Found C, 80.73; H, 11.21.

2.5. Homocyclomagnesiation of THP ether of 5,6-hepta-5,6-dien-1-ol 4 with EtMgBr in the presence of Mg metal and Cp_2TiCl_2 catalyst

Diethyl ether (10 ml), THP ether of 5,6-hepta-5,6-dien-1-ol (**4**) (0.2 g, 1.0 mmol), EtMgBr (1.3 ml, 2.0 mmol) (as a 1.5 M solution in Et_2O), Mg powder (58 mg, 2.4 mmol), and Cp_2TiCl_2 (0.12 g, 0.5 mmol) were charged into a glass reactor with stirring under argon (~0 °C). The reaction mixture was warmed-up to room temperature (20–22 °C) and stirred for 24 h. The reaction mixture was treated with a 5% solution of NH_4Cl in H_2O (10 ml). The products were extracted with diethyl ether (2 × 30 ml), the extracts were dried with $MgSO_4$, the solvent was evaporated, and the residue was chromatographed on a column (SiO_2, elution with petroleum ether/EtOAc (50/1)).

^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectral data for 2,2′-[(5Z,9Z)-tetradeca-5,9-diene-1,14-diylbis(oxy)] bistetrahydro-2H-pyran (**9**) are in agreement with the literature data [11].

*2.6. Oxidation of 1,14-tetrahydropyranyl-5Z, 9Z-dien-1,14-diol **9** with Jones reagent*

To a solution of 1,14-tetrahydropyranyl-5Z, 9Z-dien-1,14-diol (**9**) (0.2 g, 0.5 mmol) in acetone (12 ml) and CH_2Cl_2 (2 ml) at rt, Jones reagent (1.0 ml) was added dropwise. The reaction mixture was stirred at rt for 1 h, then quenched with water (5 ml), concentrated under reduced pressure to remove the excess of acetone and CH_2Cl_2, and the aqueous layer extracted with diethyl ether (3 × 10 ml). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column

chromatography using hexane/ethyl acetate = 3/1 as the elution solvent to afford (5Z,9Z)-tetradeca-5,9-dienedioic acid **10**.

*2.6.1. (5Z,9Z)-tetradeca-5.9-dienedioic acid (**10**)*

Colorless oil. Yield: 0.07 g (52%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.44 (m, 2H, HC=), 5.36 (m, 2H, HC=), 2.39 (m, 4H, CH_2COOH), 2.12 (m, 4H, CH_2CH=), 2.08 (m, 4H, CH_2CH=), 1.73 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 180.0 (C), 130.5 (CH), 128.8 (CH), 33.21 (CH_2), 27.4 (CH_2), 26.2 (CH_2), 24.5 (CH_2).

*2.7. Reaction of steroid with (5Z,9Z)-tetradeca-5.9-dienedioic acid **10***

To a mixture of steroid (cholesterol or cholestanol) (1.0 mmol), (5Z,9Z)-tetradeca-5.9-dienedioic acid (**10**) (0.51 g, 2.0 mmol) and 4-dimethylaminopyridine (18 mg, 0.15 mmol) in dichloromethane (10 ml) was added a solution of dicyclohexyl carbodiimide (0.41 g, 2.0 mmol) in 5 ml of dichloromethane under argon. The mixture was stirred at room temperature for 6 h. After removal of the precipitate by filtration, the solution was concentrated under vacuum. The crude product was purified by column chromatography (silica gel) using hexane/ethyl acetate as the elution solvent to afford dimer (**11** or **13**) and 5Z,9Z-dienoic acids of steroid (**12** or **14**).

*2.7.1. Di-(3β)-cholest-5-en-3-yl(5Z,9Z)-tetradeca-5,9-dienedioate (**11**)*

White solid. Yield: 0.15 g (15%). m.p. 99–101 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.38 (m, 4H, HC=), 5.36 (m, 2H, H-6), 4.63 (m, 2H, H-3), 2.30 (m, 4H, CH_2COO), 2.10 (m, 4H, CH_2CH=), 2.09 (m, 4H, CH_2CH=), 1.69 (m, 4H, CH_2), 1.04 (s, 6H, H-19), 0.94 (d, J = 6.4 Hz, 6H, H-21), 0.89 (d, J = 6.4 Hz, 12H, H-26 and H-27), 0.69 (s, 6H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 173.1 (C), 139.7 (C), 130.2 (CH), 129.1 (CH), 122.6 (CH), 73.8 (CH), 56.7 (CH), 56.2 (CH), 50.0 (CH), 42.3 (C), 39.7 (CH_2), 39.5 (CH_2), 38.2 (CH_2), 37.0 (CH_2), 36.6 (C), 36.2 (CH_2), 35.8 (CH), 34.1 (CH_2), 31.9 (CH_2), 31.9 (CH), 28.2 (CH_2), 28.0 (CH), 27.8 (CH_2), 27.3 (CH_2), 26.6 (CH_2), 24.9 (CH_2), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.0 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3).

*2.7.2. (5Z,9Z)-14-[(3β)-cholest-5-en-3-yloxy]-14-oxotetradeca-5,9-dienoic acid (**12**)*

White waxy solid. Yield: 0.35 g (57%), $[\alpha]_D^{20}$ – 22.1 (*c* 3.00, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.43 (m, 2H, CH=), 5.38 (m, 1H, H-6), 5.37 (m, 2H, CH=), 4.63 (m, 1H, H-3), 2.38 (m, 2H, CH_2COOH), 2.31 (m, 2H, CH_2COO), 2.12 (m, 4H, CH_2CH=), 2.08 (m, 4H, CH_2CH=), 1.72 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 1.04 (s, 3H, H-19), 0.93 (d, J = 6.4 Hz, 3H, H-21), 0.88 (d, J = 6.4 Hz, 6H, H-26 and H-27), 0.69 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 178.96 (C), 173.4 (C), 139.7 (C), 130.4 (CH), 130.1 (CH), 129.1 (CH), 128.8 (CH), 122.6 (CH), 73.8 (CH), 56.7 (CH), 56.1 (CH), 50.0 (CH), 42.3 (C), 39.7 (CH_2), 39.5 (CH_2), 38.1 (CH_2), 36.9 (CH_2), 36.6 (C), 36.2 (CH_2), 35.8 (CH), 34.1 (CH_2), 33.3 (CH_2), 31.9 (CH_2), 31.9 (CH), 28.2 (CH_2), 28.0 (CH), 27.8 (CH_2), 27.4 (CH_2), 27.3 (CH_2), 26.6 (CH_2), 26.5 (CH_2), 25.0 (CH_2), 24.6 (CH_2), 24.3 (CH_2), 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.0 (CH_2), 19.4 (CH_3), 18.7 (CH_3), 11.9 (CH_3). MALDI TOF: *m*/*z* 645.448 ([M+Na]$^+$, calcd 645.486). Anal. Calcd for $C_{41}H_{66}O_4$: C, 79.05; H, 10.68. Found C, 78.81; H, 10.64.

*2.7.3. Di-(3β,5α)-cholestan-3-yl(5Z,9Z)-tetradeca-5,9-dienedioate (**13**)*

White solid. Yield: 0.12 g (12%). m.p. 78–80 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.40 (m, 2H, HC=), 5.36 (m, 2H, HC=), 4.72 (m, 1H, H-3), 2.28 (t, J = 7.5 Hz, 2H, CH_2COO), 2.10 (m, 4H, CH_2CH=), 2.09 (m, 4H, CH_2CH=), 1.68 (m, 4H, CH_2), 0.91 (d, J = 6.5 Hz, 6H, H-21), 0.88 (d, J = 6.5 Hz, 12H, H-26 and H-27), 0.84 (s, 6H, H-19), 0.67 (s, 6H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 173.2 (C), 130.2 (CH), 129.1 (CH), 73.5 (CH), 56.4 (CH), 56.3 (CH), 54.2 (CH), 44.7 (CH), 42.6 (C), 39.9 (CH_2), 39.5 (CH_2), 36.8 (CH_2), 36.2 (CH_2), 35.8 (C), 35.5 (CH), 35.5 (CH), 34.1 (CH_2), 34.1 (CH_2), 31.9 (CH_2), 28.6 (CH_2), 28.2 (CH_2), 28.0 (CH), 27.5 (CH_2), 27.3 (CH_2), 26.6 (CH_2), 25.0 (CH_2), 24.2 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.2 (CH_2), 18.7 (CH_3), 12.2 (CH_3), 12.1 (CH_3).

*2.7.4. (5Z,9Z)-14-[(3β,5α)-cholestan-3-yloxy]-14-oxotetradeca-5,9-dienoic acid (**14**)*

White waxy solid. Yield: 0.36 g (58%), $[\alpha]_D^{20}$ + 10.7 (*c* 1.37, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.41 (m, 2H, CH=), 5.35 (m, 2H, CH=), 4.72 (m, 1H, H-3), 2.37 (t, J = 7.5 Hz, 2H, CH_2COOH), 2.29 (t, J = 7.5 Hz, 2H, CH_2COO), 2.11 (m, 4H, CH_2CH=), 2.09 (m, 4H, CH_2CH=), 1.71 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 0.91 (d, J = 6.5 Hz, 3H, H-21), 0.88 (d, J = 6.5 Hz, 6H, H-26 and H-27), 0.83 (s, 3H, H-19), 0.66 (s, 3H, H-18). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 179.6 (C), 173.5 (C), 130.4 (CH), 130.1 (CH), 129.1 (CH), 128.8 (CH), 73.7 (CH), 56.4 (CH), 56.3 (CH), 54.2 (CH), 44.7 (CH), 42.6 (C), 39.9 (CH_2), 39.5 (CH_2), 36.8 (CH_2), 36.2 (CH_2), 35.8 (C), 35.5 (CH), 35.5 (CH), 34.2 (CH_2), 34.1 (CH_2), 33.4 (CH_2), 31.9 (CH_2), 28.6 (CH_2), 28.3 (CH_2), 28.0 (CH), 27.5 (CH_2), 27.3 (CH_2), 27.3 (CH_2), 26.6 (CH_2), 26.5 (CH_2), 25.0 (CH_2), 24.6 (CH_2), 24.2 (CH_2), 23.9 (CH_2), 22.8 (CH_3), 22.6 (CH_3), 21.2 (CH_2), 18.7 (CH_3), 12.2 (CH_3), 12.1 (CH_3). MALDI TOF: *m*/*z* 647.469 ([M+Na]$^+$, calcd 647.502). Anal. Calcd for $C_{41}H_{68}O_4$: C, 78.79; H, 10.97. Found C, 78.62; H, 10.94.

2.8. Biological in vitro investigations

2.8.1. DNA topoisomerase I assay

The inhibitory activity of acids were determined using the Topoisomerase I Drug Screening Kit TG-1018-2, (Topogen, USA) (the tested compound was added before topoisomerase I). The relaxation of supercoiled DNA under the action of topoisomerase I was carried out as follows: the reaction mixture (20 μl) containing 0.25 μg of the DNA plasmid pHOT (TopoGen, USA), 1 unit of recombinant topoisomerase I (TopoGen, USA), and the tested compound: dienoic acid was incubated in the buffer (35 mM Tris–HCl, pH 8.0; 72 mM KCl, 5 mM MgCl, 5 mM dithiothreitol, 5 mM spermidine, and 0.01% bovine serum albumin) for 30 min at 37 °C using a Biosan thermostat (Latvia). The tested compound was introduced in the reaction mixture prior to the addition of the enzyme topoisomerase I. The inhibiting action on topoisomerase I was monitored using the alkaloid camptothecin (TopoGEN, USA). The reaction was terminated by adding sodium dodecyl sulfate up to a concentration of 1%. After addition of a solution (5 mg/ml) of proteinase K (Sigma Chemical Co., USA) (1:10), the reaction mixture was incubated for 15 min at 37 °C. A 0.1% solution of bromophenol blue (1:10) was added and the samples were electrophoresed in the presence and absence of ethidium bromide. The reaction products were separated in a 1% agarose gel (3 V/cm) for 2–3 h. After the electrophoresis without ethidium bromide, the gels were treated with a solution of ethidium bromide (0.5 μg/ml). The gels were visualized in the UV light in a Gel Doc™ EZ System (BioRad, USA). The possible action of the tested compounds on supercoiled DNA was checked by performing the reaction without topo I, the tested compounds being added in the same concentrations as in the reaction with the enzyme.

2.8.2. The relaxation reaction of plasmid DNA

To perform the relaxation reaction of plasmid DNA, a common mixture (20 μl) was prepared from the reaction components in stoichiometric amounts without addition of inhibiting compounds and topo I, as described in the Experimental. As the negative control, a supercoiled plasmid sample was taken and neither test

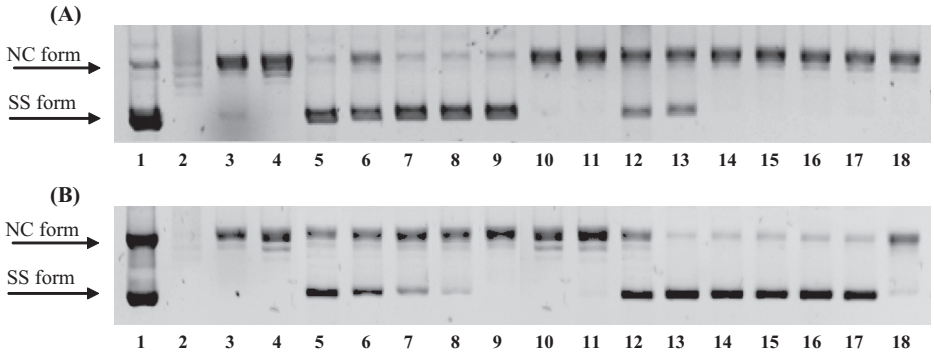


Fig. 1. Electrophoregram of the products of *in vitro* relaxation of supercoiled plasmid DNA under the action of topoisomerase I (Topogen, USA) in the presence of acids **12**, **7a** (A) and **7b**, **14** (B). (A) **1**. Supercoiled plasmid DNA (pHOT1). **2**. Relaxed plasmid DNA (pHOT-1). **3**. Supercoiled plasmid DNA + topoisomerase I + camptothecin (10 μM) – positive control (10 μM). **4**. Supercoiled plasmid DNA + topoisomerase I + DMSO (3%). **5**–**11**. Supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **12** at various concentrations (**5** – 10 μM, **6** – 1 μM, **7** – 0.1 μM, **8** – 0.08 μM, **9** – 0.06 μM, **10** – 0.04 μM, **11** – 0.02 μM). **12**–**18**. Supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **7a** at various concentrations (**12** – 10 μM, **13** – 1 μM, **14** – 0.1 μM, **15** – 0.08 μM, **16** – 0.06 μM, **17** – 0.04 μM, **18** – 0.02 μM). (B) **1**. Supercoiled plasmid DNA (pHOT1). **2**. Relaxed plasmid DNA (pHOT-1). **3**. Supercoiled plasmid DNA + topoisomerase I + camptothecin (10 μM) – positive control (10 μM). **4**. Supercoiled plasmid DNA + topoisomerase I + DMSO (3%). **5**–**11**. Supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **7b** at various concentrations (**5** – 10 μM, **6** – 1 μM, **7** – 0.1 μM, **8** – 0.08 μM, **9** – 0.06 μM, **10** – 0.04 μM, **11** – 0.02 μM). **12**–**18**. Supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **14** at various concentrations (**12** – 10 μM, **13** – 1 μM, **14** – 0.1 μM, **15** – 0.08 μM, **16** – 0.06 μM, **17** – 0.04 μM, **18** – 0.02 μM).

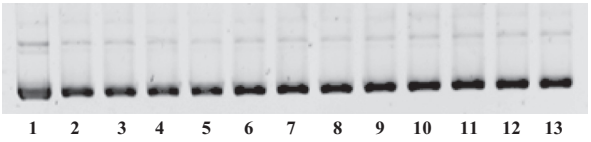


Fig. 2. Modification of gel electrophoretic mobility of pHOT1 supercoiled plasmid DNA when incubated (2 h, 37 °C) with various concentrations of acids **7a**,**b**, **12** и **14**. Concentrations (in μM) are as follows: (lane 1) untreated pHOT1 supercoiled plasmid DNA; (lanes 2–4, incubated with **7a**): 10, 1, 0.1; (lanes 5–7, incubated with **7b**): 10, 1, 0.1; (lanes 8–10, incubated with **12**): 10, 1, 0.1; (lanes 11–13, incubated with **14**): 10, 1, 0.1. The top and the bottom bands correspond to open circular form, covalently closed circular form, and linear conformation form of plasmids, respectively.

compounds nor topoisomerase I was added. The same sample with addition of topo I and camptothecin served as the positive control. To other samples, topo I and solutions of test compounds were added (Fig. 2).

3. Results and discussion

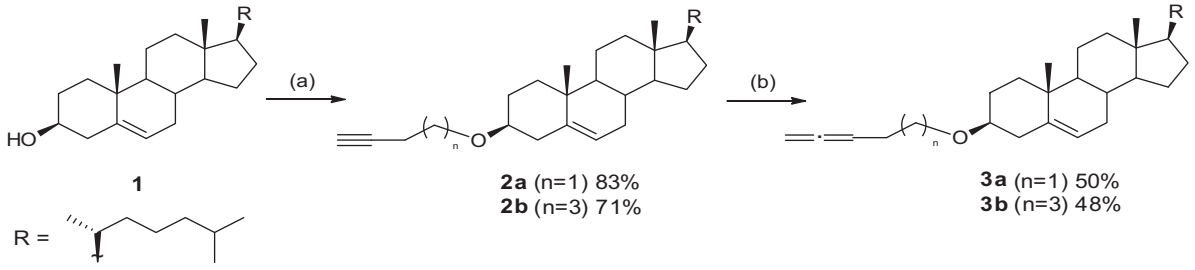
First, previously undescribed 1,2-dienes **3a** and **3b** were synthesized in two steps. The first step was the synthesis of ethers **2a** and **2b** by the reaction of cholesterol **1** with alkynols (but-3-yn-1-ol, hex-5-yn-1-ol) in the presence of Montmorillonite K-10 (refluxing in chloroform for 7 days) [14]. The Mannich reaction of synthesized ethers **2a** and **2b** with paraformaldehyde afforded target 1,2-dienes **3a** and **3b** (Scheme 2) [15].

According to the outlined strategy of the synthesis of 5Z,9Z-dienoic acids, cholesterol allene ethers **3a** and **3b** were subjected to cross-cyclomagnesiation with the tetrahydropyran ether of hepta-5,6-dien-1-ol **4** on treatment with EtMgBr in the presence of magnesium and the Cp_2TiCl_2 catalyst (10 mol%) under the conditions: **3a**(**3b**):**4**:EtMgBr:Mg:[Ti] = 1:3:8:12:0.1, Et_2O, r.t., 24 h to give magnesacyclopentanes **5**, which underwent acid hydrolysis to give dienes **6a** and **6b** (Scheme 3). It is noteworthy that the yield of the target products of cross-cyclomagnesiation of cholesterol 1,2-diene ethers increases as the allene group moves away from the steroid skeleton. The Jones oxidation of compounds **6a** and **6b** affords the target 5Z,9Z-dienoic acids **7a** and **7b** in ~50% yields [16].

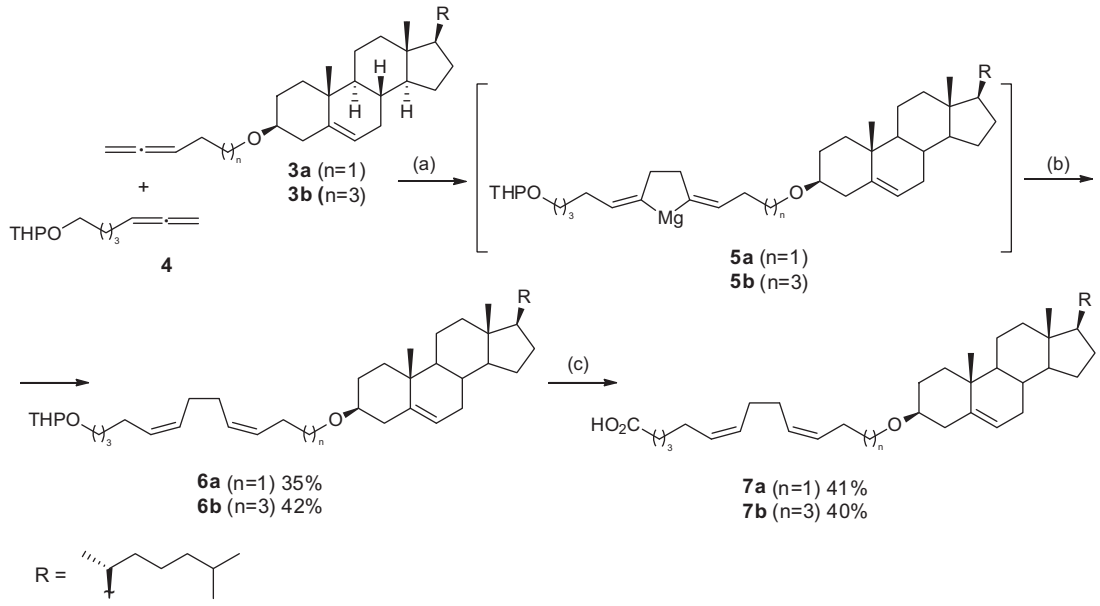
For the other approach to the synthesis of 5Z,9Z-dienoic acid derivatives, we developed a two-step method for the synthesis of tetradeca-5Z,9Z-diene-1,14-dicarboxylic acid. The first step included homo-cyclomagnesiation of the tetrahydropyran ether of 5,6-hepta-5,6-dien-1-ol **4** on treatment with EtMgBr in the presence of magnesium and the Cp_2TiCl_2 catalyst (5 mol%). The acid hydrolysis of magnesacyclopentane **8** formed *in situ* gives 1,14-bis-tetrahydropyranyl-5Z,9Z-diene-1,14-diol **9** in 74% yield. The Jones oxidation of the latter furnishes (5Z,9Z)-tetradeca-5,9-dienedioic acid **10** in 52% yield (Scheme 4).

The subsequent catalytic esterification of steroids with (5Z,9Z)-tetradeca-5,9-dienedioic acid **10** using DCC/DMAP afforded the desired steroid 5Z,9Z-dienoic acids **12** and **14** in ~55–60% yields, together with symmetric dimers **11** and **13**, the yields of which did not exceed 15% (Scheme 5) [17].

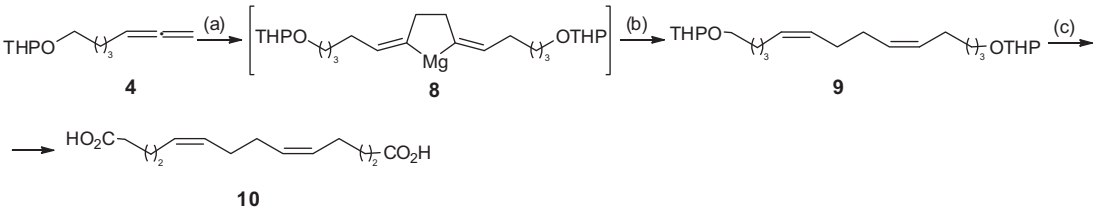
The structure of synthesized compounds **6**, **7** and **10**–**14** was reliably characterized by one-dimensional (^1H, ^{13}C, Dept 135) and two-dimensional (HSQC, HMBC, and HH COSY, NOESY) NMR



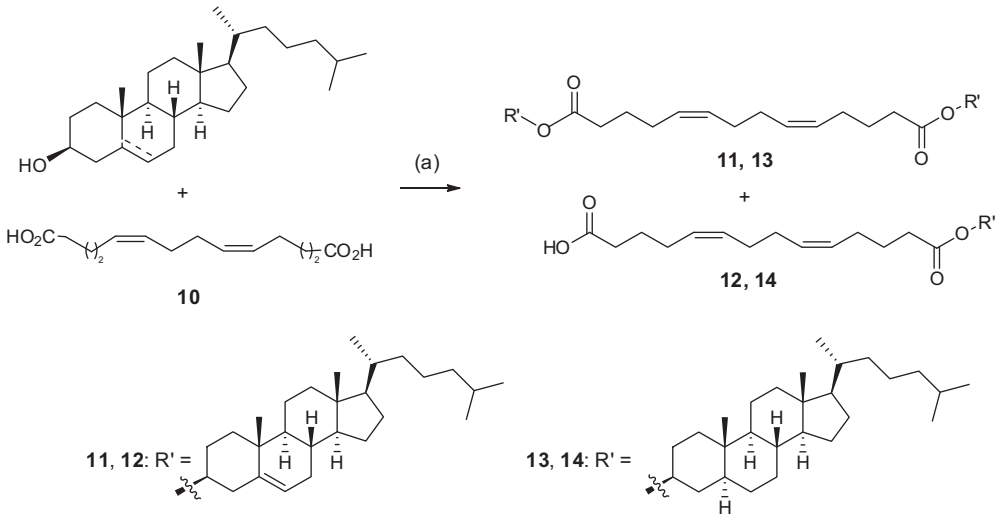
Scheme 2. Synthesis of compounds **3a** and **3b**. (a): Montmorillonite K-10, $CHCl_3$, but-3-yn-1-ol (hex-5-yn-1-ol); (b): formaldehyde, CuI, diisopropylamine, dioxane, reflux.



Scheme 3. Reaction of cross-cyclomagnesiation. (a): EtMgBr, Mg, Cp_2TiCl_2 (10 mol%), diethyl ether; (b): H_3O^+; (c): H_2CrO_4/H_2SO_4, acetone, CH_2Cl_2.



Scheme 4. Synthesis of (5Z,9Z)-tetradeca-5,9-dienedioic acid. (a): EtMgBr, Mg, Cp_2TiCl_2 (5 mol%), diethyl ether; (b): H_3O^+; (c): H_2CrO_4/H_2SO_4, acetone, CH_2Cl_2.



Scheme 5. Synthesis of 5Z,9Z-dienoic acids of cholesterol **12** and cholestanol **14**. (a): DMAP, DCC, CH_2Cl_2.

procedures. The presence of high-field signals for allylic carbon atoms with δC of 27 ppm in the ^{13}C NMR spectra attests to the *cis*-configuration of substituents at the double bonds in compounds **6**, **7** and **10**–**14** [18].

The new semisynthetic derivatives of steroid 5Z,9Z-dienoic acids **7a**,**b**, **12**, and **14** were tested *in vitro* for the inhibitory activity against human topoisomerase I in the relaxation of supercoiled plasmid DNA under standard conditions (Fig. 1). The known hTopI inhibitor, camptothecin, was used as the reference compound in all experiments.

Under the action of topoisomerase I, the supercoiled plasmid DNA relaxes to give a set of topoisomers. The method of determination of topoisomerase I inhibitory activity is based on high sensitivity of the electrophoretic mobility of various forms of DNA

(initial form and enzyme-induced relaxation products) to differences in the duplex conformation: supercoiled and circular plasmids. The introduction of a compound inhibiting the topoisomerase I activity disrupts the relaxation process, which may decrease the number of topoisomers formed, increase the percentage of the circular plasmid and result in the presence of residual supercoiled plasmid DNA. The inhibition of topoisomerase I may be specific (the formation of long-lived covalent complexes between the compound, the enzyme, and DNA) or non-specific (inhibition of any other step of the topo I catalytic cycle, including direct interaction with DNA or the enzyme). In the case of specific (poisoning) inhibition mechanism, stabilization of the DNA–topoisomerase I covalent complex by the inhibitor prevents ligation of the DNA duplex and leads to accumulation of single DNA breaks, which violates vital processes in the cell. A characteristic sign of this type of inhibition is accumulation of the open circular form of plasmid DNA.

The decrease in the concentration of 5Z,9Z-dienoic acids introduced in the reaction from 10 μM to 0.02 μM caused a gradual increase in the number of topoisomers formed and increase in the percentage of the open circular plasmid form, indicating the activation of relaxation process, that is, increase in the topo I activity (Fig. 1). In the absence of test compounds or camptothecin, this effect was not observed (lane 4, Fig. 1A and B). In the presence of camptothecin, a noticeable inhibition of the relaxation was observed upon the camptothecin addition in a 10 μM concentration, which was manifested as retention of the residual amounts of supercoiled DNA compared with the negative control: supercoiled plasmid without addition of the enzyme or inhibitor (SS form). Acids **7a** and **7b** behaved almost identically in the concentration range studied; they started to inhibit topo I at lower concentrations (>1 μM) than camptothecin. Compounds **12** and **14** showed a somewhat higher inhibitory activity against topo I, as the increase in the number of topoisomers started at lower concentrations of these compounds (0.06 μM and 0.02 μM, respectively). At the 0.02 μM concentration of acid **14**, only some of the supercoiled DNA was relaxed, which was not observed upon the action of acids **7a**,**b** and **12**. Judging by the residual amount of the unreacted supercoiled plasmid, the inhibitory activity of acid **14** was higher than the activity of any other compound in the same concentration range.

Since some dienoic acid derivatives are known to interact with DNA [19], the electrophoretic mobility of DNA molecules observed in our experiments could have been determined by not only a change in the activity of topo I but also direct influence of dienoic acids on the conformation of the DNA molecule. Therefore, it was necessary to elucidate the concentration range in which the test compounds can themselves affect the electrophoretic mobility of DNA. For this purpose, the effect of acids **7a**,**b**, **12**, and **14** on the mobility of supercoiled DNA was measured in the absence of DNA-dependent topo I enzyme. Fig. 2 shows the electrophoregram for this experiment. The results presented in Fig. 2 indicate that the acids in question do not affect the DNA mobility at concentrations of 10, 1, and 0.1 μM. The key results on topo I inhibition were obtained with test compound concentrations below 10 μM; therefore, it is obvious that the obtained experimental data are correct.

4. Conclusions

Thus, two synthetic routes to 5Z,9Z-dienoic acids containing a steroid skeleton in the molecule were proposed, one based on cross-cyclomagnesiation of allene derivatives of cholesterol with tetrahydropyran ether of hepta-5,6-dien-1-ol on treatment EtMgBr and catalyzed by Cp_2TiCl_2 and the other based on steroid esterification reaction with (5Z,9Z)-tetradeca-5,9-dienedioic acid.

Among the synthesized steroid derivatives, acids **12** and **14** prepared by the latter approach exhibited a two orders of magnitude higher activity against human topoisomerase I. In our opinion, the developed reactions bear extensive synthetic potential that would make it possible to synthesize a broad range of 5Z,9Z-dienoic acids with substituents of various nature. Estimation of the topo I inhibitory action and elucidation of the structure–activity relationships in the series of 5*Z*,9*Z*-dienoic acids or their semisynthetic derivatives would facilitate the targeted search for new active inhibitors of this enzyme potentially suitable as pharmaceutical drugs.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2015.08.006.

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