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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

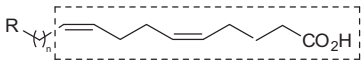
Two approaches to stereoselective synthesis of steroid 5Z,9Z-dienoic acids were developed, the first one being based on the cross-cyclomagnesiation of 2-(hepta-5,6-dien-1-yloxy)tetrahydro-2H-pyran and 1,2-diene cholesterol derivatives on treatment with EtMgBr catalyzed by Cp_2TiCl_2, while the other 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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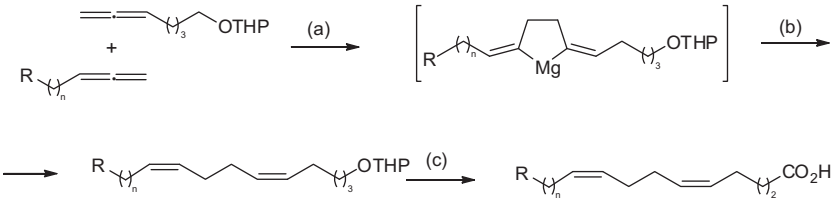

Scheme 1. Method for the synthesis of 5Z,9Z-dienoic acids. (a): EtMgBr, Mg, Cp_2TiCl_2 (10 mol%), diethyl ether; (b): H_3O^+; (c): H_2CrO_4/H_2SO_4, acetone, CH_2Cl_2.

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