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Synthesis and radical scavenger properties of novel spirochromenes derived from steroid sapogenins

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ABSTRACT

Tandem aldol condensation between steroid sapogenins and hydroxylated benzaldehydes afforded steroidal spirochromenes. Compounds that bear a phenolic hydroxyl group at position C-6′, obtained by a reaction with 2,5-dihydroxybenzaldehyde, showed approximately 80% of maximal radical scavenging activity in the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay at 288 nM. In contrast, the starting steroid sapogenins and the spirochromenes without a phenolic group in the side chain proved to be inactive.

1. Introduction

Steroids play a paramount role in life processes. The wide variety of naturally occurring members of this family includes sex and adrenocortical hormones [1], cytotoxic compounds [2–4], ecdysteroids [5] and plant growth promoting substances [6–8] amongst many others. In addition, a large battery of synthetic steroidal drugs has been developed for the treatment of different diseases [9]. However, in spite of the vast number of biologically active steroids that has been described, to the best of our knowledge, a limited number of steroids has been reported to exert a significant antioxidative activity [10–17].

Oxidative stress is associated to a wide variety of health disorders [18–20]. Consequently, the experimental and theoretical study of naturally occurring and synthetic compounds with antioxidant activity has concentrated an increased attention in the past years [21–27]. The protective effect against oxidative stress exerted by naturally occurring antioxidants like α-tocopherol (**1**) (*also referred as vitamin E*) and the phenolic carotenoid 3,3′-dihydroxyisorenieratene (**2**), as well as the effect of Trolox (**3**), a water soluble synthetic analogue of vitamin E (Fig. 1), are well documented [24,25] and the role of the phenolic hydroxyl groups present in these compounds and other phenolic antioxidants, such as resveratrol and nordihydroguaiaretic acid, is well understood [23,26,27].

In connection with our project on the study of reactivity of the spiroketal side chain of steroidal sapogenins [28–31], we have recently described that the reaction of sarsasapogenin acetate (**4**) and salicylaldehyde produces the unreported diastereomeric steroidal spirochromenes **22*R*-5** and **22*S*-5** (Scheme 1) [32]. Considering the structural similarity between the pharmacophoric cores of compounds **1**–**3**, and the spirochromene moiety present in our recently reported spirochromenes **22*R*-5** and **22*S*-5**, we have decided to embark in additional synthetic efforts to generate new members of this novel family of compounds that may show antioxidant activity.

Herein we report the synthesis and characterization of two novel steroidal spirochromenes bearing a phenolic hydroxyl group at position C-6′. The hereto unreported compounds, that showed interesting properties as radical scavengers in the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) test [33], can be easily obtained by tandem aldol condensation-spiroketalization between steroid sapogenins and 2,5-dihydroxybenzaldehyde. In addition, steroidal spirochromenes without the phenolic hydroxyl group were prepared in order to be employed as references in the radical scavenging activity test.

2. Experimental

Reactions were monitored by TLC on ALUGRAM®SIL G/UV254 plates from MACHEREY-NAGEL. Chromatographic plates were sprayed with a 1% solution of vanillin in 50% $HClO_4$ and heated until color developed. Melting points were measured on a

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0039-128X/

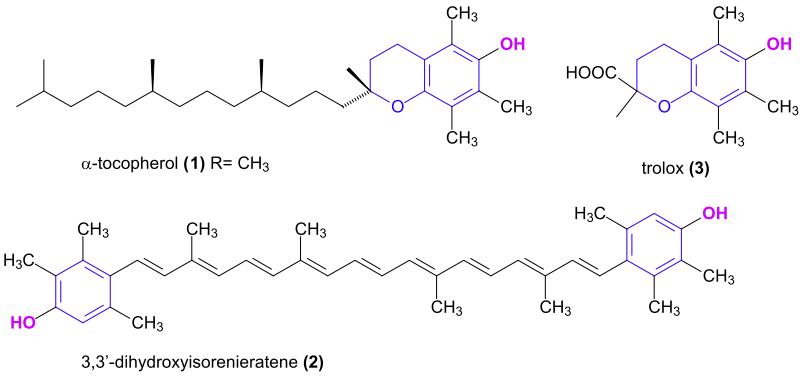
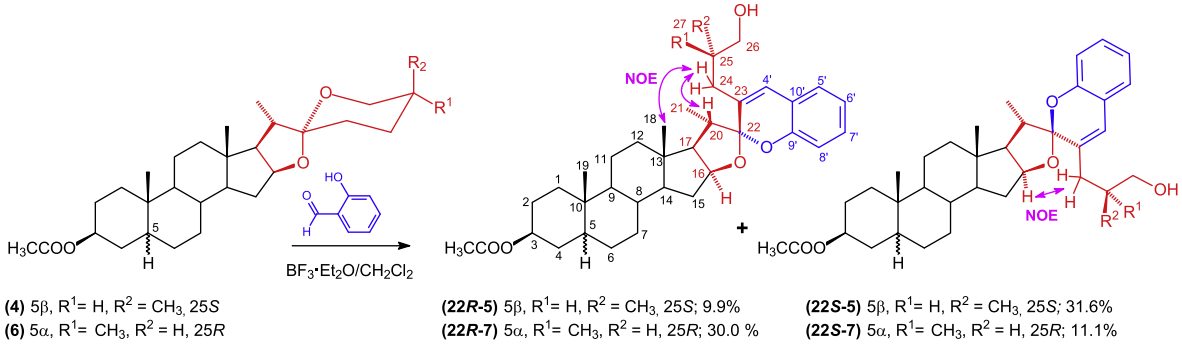


Fig. 1. Some antioxidants.



(4) 5β, R^1= H, R^2 = CH_3, 25*S*
(6) 5α, R^1= CH_3, R^2 = H, 25*R*

(22*R*-5) 5β, R^1= H, R^2 = CH_3, 25*S*; 9.9%
(22*R*-7) 5α, R^1= CH_3, R^2 = H, 25*R*; 30.0 %

(22*S*-5) 5β, R^1= H, R^2 = CH_3, 25*S;* 31.6%
(22*S*-7) 5α, R^1= CH_3, R^2 = H, 25*R*; 11.1%

Scheme 1. Aldol reaction between steroids sapogenins and salicylaldehyde.

Melt-Temp II apparatus. Mass spectra were registered in a Thermo-Electron spectrometer model DFS (Double Focus Sector). NMR spectra were recorded in $CDCl_3$ solution in a Varian INOVA 400 spectrometer using the solvent signal 7.26 ppm for ^1H and 77.00 ppm for ^{13}C as references. All 2D NMR spectra were recorded using the standard pulse sequences and parameters recommended by the manufacturer and were processed employing the MestreNova NMR processing program [See http://mestrelab.com/]. NMR signals assignments were carried out with the aid of a combination of 1D and 2D NMR techniques that included ^1H, ^{13}C, ^1H–^1H COSY, Nuclear Overhauser Effect Spectroscopy (NOESY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC).

General procedure for the preparation of spirochromenes

$BF_3{\cdot}Et_20$ (6 mL) was added to a solution of the steroid sapogenin (1 mmol) and the aldehyde (2 mmol) in CH_2Cl_2 (30 mL). And the mixture was stirred at room temperature until the starting material was consumed (~24 h). The reaction mixture was carefully poured into ice and extracted with ethyl acetate (2 × 30 mL). The organic layer was washed with water (10 × 15 mL) and a saturated solution of NaCl (1 × 15 mL), dried (anh. Na_2SO_4) and evaporated. The obtained oil was purified in a chromatographic column packed with silica gel to afford the desired spirochromenes.

Reaction of sarsasapogenin acetate (**4**) (458 mg, 1 mmol) with salicylaldehyde (244 mg, 2 mmol) afforded (22*R*,25*S*)-16β:22-epoxy-22,2′-spiro-[chromene]-5β-cholestan-3β,26-diol 3-monoacetate (**22*R*-5**). Yield 54.5 mg, (9.7%) (light yellow syrup). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.19–7.07 (m, 1H, H-7′), 7.02 (dd, *J* = 7.8, 1.7 Hz, 1H, H-5′), 6.87 (ddd, *J* = 7.4, 6.2, 1.3 Hz, 2H, H-6′and H-8′), 6.47 (s, 1H, H-4′), 5.07 (p, *J* = 2.7 Hz, 1H, H-3α), 4.73 (ddd, *J* = 11.0, 8.1, 5.0 Hz, 1H, H-16α), 3.62 (dd, *J* = 11.0, 4.6 Hz, 1H, H-26a), 3.45 (dd, *J* = 10.9, 4.9 Hz, 1H, H-26b), 2.54 (t, *J* = 7.1 Hz, 1H, H-20β), 2.36 (dd, *J* = 14.0, 6.8 Hz, 1H, H-24a), 2.15–2.07 (m, 2H, H-17 and H-24b) 2.04 (s, 3H, CH_3 acetyl), 1.03 (d, *J* = 6.8 Hz, 3H, H-21), 0.99 (d, *J* = 7.7 Hz, 3H, H-27), 0.98 (s, 3H, H-19), 0.89 (s, 3H, H-18). ^{13}C NMR (100.52 MHz) δ (ppm): 30.6 C-1, 24.9 C-2, 70.6 C-3, 31.6 C-4, 37.2 C-5, 26.4 C-6, 26.3 C-7, 35.0 C-8, 39.9 C-9, 35.0 C-10, 20.9 C-11, 40.3 C-12, 41.2 C-13, 56.1 C-14, 29.7 C-15, 82.6 C-16, 60.6 C-17, 16.7 C-18, 23.8 C-19, 40.3 C-20, 14.7 C-21, 113.5 C-22, 130.9 C-23, 35.1 C-24, 35.7 C-25, 66.9 C-26, 17.1 C-27, 125.4 C-4′, 126.0 C-5′, 120.9 C-6′, 128.4 C-7′, 115.5 C-8′, 151.3 C-9′, 120.8 C-10′, 21.5 CH_3 acetyl, 170.7 C=O acetyl. MS (EI, 70 eV) *m/z* (%) 562(5.4) M^+, 284(10.6), 269(24.7), 255(14.9), 243(21.7), 220(14.4), 219(100), 202(10.5), 201(63.5), 173(10.4), 161(14.7), 160(16.9), 159(29.2), 147(15.1), 145(13.9), 133(12.3), 131(14.8), 122(14.3), 121(15.1), 119(11.4), 107(27.8), 105(16.6), 95(14.3), 93(21.1), 91(15.7), 81(17.5), 79(15.4), 67(11.9), 55(11.1).

and

(22*S*,25*S*)-16β:22-epoxy-22,2′-spiro-[chromene]-5β-cholestan-3β,26-diol 3-monoacetate (**22*S*-5**). Yield 178.1 mg (31.6%) (light yellow syrup). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.16–7.09 (m, 1H, H-7′), 7.06 (dd, *J* = 7.7, 1.6 Hz, 1H, H-5′), 6.92–6.87 (m, 2H H-6′ and H-8′), 6.48 (s, 1H, H-4′), 5.13–5.01 (m, 1H, H-3a), 4.68 (ddd, *J* = 11.0, 8.1, 5.0 Hz, 1H, H-16α), 3.57 (dd, *J* = 10.7, 5.9 Hz, 1H, H-26a), 3.51 (dd, *J* = 10.7, 5.2 Hz, 1H, H-26b), 2.57 (p, *J* = 6.9 Hz, 1H, H-20β), 2.35 (ddd, *J* = 14.7, 7.7, 1.1 Hz, 1H, H-24a), 2.18–2.10 (m, 1H, H-24b), 2.05 (s, 3H, CH_3 acetyl), 1.06 (d, *J* = 6.8 Hz, 3H, H-21), 0.99 (d, *J* = 6.2 Hz, 3H, H-27) 0.99 (s, 3H, H-19), 0.89 (s, 3H, H-18). ^{13}C NMR (100.52 MHz) δ (ppm): 30.6 C-1, 24.9 C-2, 70.6 C-3, 31.6 C-4, 37.2 C-5, 26.4 C-6, 26.3 C-7, 35.0 C-8, 39.9 C-9, 35.0 C-10, 20.9 C-11, 40.3 C-12, 41.1 C-13, 56.1 C-14, 30.7 C-15, 82.4 C-16, 60.8 C-17, 16.6 C-18, 23.8 C-19, 39.4 C-20, 14.8 C-21, 112.8 C-22, 130.8 C-23, 36.8 C-24, 35.1 C-25, 67.5 C-26, 17.4 C-27, 125.8 C-4′, 126.1 C-5′, 121.1 C-6′, 128.4 C-7′, 115.8 C-8′, 151.2 C-9′, 121.2 C-10′, 21.5 CH_3 acetyl, 170.7 C=O acetyl. MS (EI, 70 eV) *m/z* (%) 562(5.5) M^+, 284(10.4),