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Short Report

Synthesis and Biological Activity of Three New 5α-Hydroxy Spirostanic Brassinosteroid Analogues

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Três novos análogos espirostânicos de brassinosteróides foram sintetizados pela primeira vez a partir de diosgenina: (25R)-2α,3α-epoxi-5α-hidroxiespirostan-6-ona (**3**), (25R)-2β,3α,5α-triidroxi-espirostan-6-ona (**5**) e (25R)-2β-metoxi-3α,5α-diidroxiespirostan-6-ona (**6**). A substância **3** mostrou acentuada atividade promotora de crescimento vegetal no bioensaio de elongação do hipocótilo e expansão do cotilédone de rabanete, enquanto a substância **6** mostrou-se fitotóxica.

Three new spirostanic brassinosteroid analogues have been synthesized for the first time from diosgenin: (25R)-2α,3α-epoxy-5α-hydroxyspirostan-6-one (**3**), (25R)-2β,3α,5α-trihydroxyspirostan-6-one (**5**) and (25R)-2β-methoxy-3α,5α-dihydroxyspirostan-6-one (**6**). In the radish hypocotyl elongation and cotyledon expansion bioassay compound **3** showed plant growth promoting activity whereas **6** was shown to be phytotoxic.

Keywords: steroids, brassinosteroid analogues, diosgenin

Introduction

Brassinosteroids (BS) are steroidal phytohormones with high plant growth-promoting and anti-stress effects. [1] The effects of BS on crop yield and stress tolerance in agronomical important plants had been evaluated in several greenhouse and field trials. [2]

However, the low abundance of BS in natural sources and the difficulty of their synthesis have stimulated several workers to employ more readily available analogues in field trials. Previous reports indicate that spirostans can serve as raw materials for the synthesis of a variety of compounds with plant growth-promoting activity. [3]

Taking into account the different effects showed for some analogues of BS containing additional 5α-hydroxy group in addition to the 6-keto function [4, 5] and in order to enlarge studies of the effects on bioactivity elicited by introducing a hydroxyl group at C-5 with α-configuration in the molecule, in this paper we report the synthesis and bioactivity of three new 5α-hydroxy spirostanic BS analogues from diosgenin.

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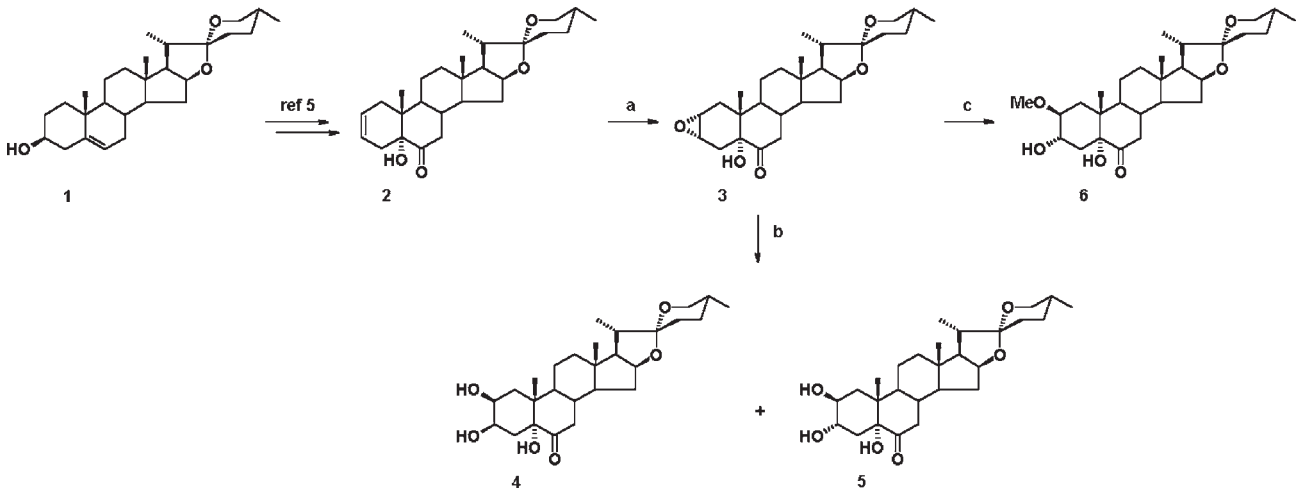
Results and Discussion

Synthesis and structural elucidation

The synthesis of the new compounds (**3, 5** and **6**) is summarized in Scheme 1. Key compound **2** was obtained in six steps from diosgenin [6] by a similar procedure developed for the synthesis of 5-hydroxy-5α-stigmasta-2,22-dien-6-one from stigmasterol. [5]

Treatment of olefin **2** with 3-chloroperoxybenzoic acid (MCPBA) in methylene chloride at room temperature for 1 h in the dark afforded the α-epoxide **3** as the major product (68%). Its structure was established as a result of analysis of ^{1}H-^{1}H-COSY spectrum, in which the signals for the protons at C-2 and C-3 appear at the characteristic positions for α-epoxy derivatives at δ 3.27 ppm and 3.56 ppm, respectively. ^{13}C NMR analysis of compound **3** was also consistent with the assigned stereochemistry (see table 1 in experimental section).

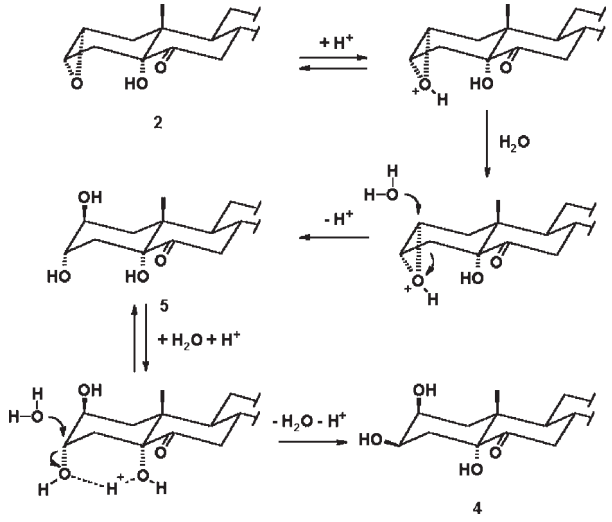
Target compounds **5** and **6** were obtained through a bifurcation of this synthetic route. Acid hydrolysis of epoxide **3** with perchloric acid/acetone afforded, besides 7% of *cis*-diol **4**, the expected *trans*-diol **5** as the major compound in 85% yield. Formation of 2β,3β,5α-



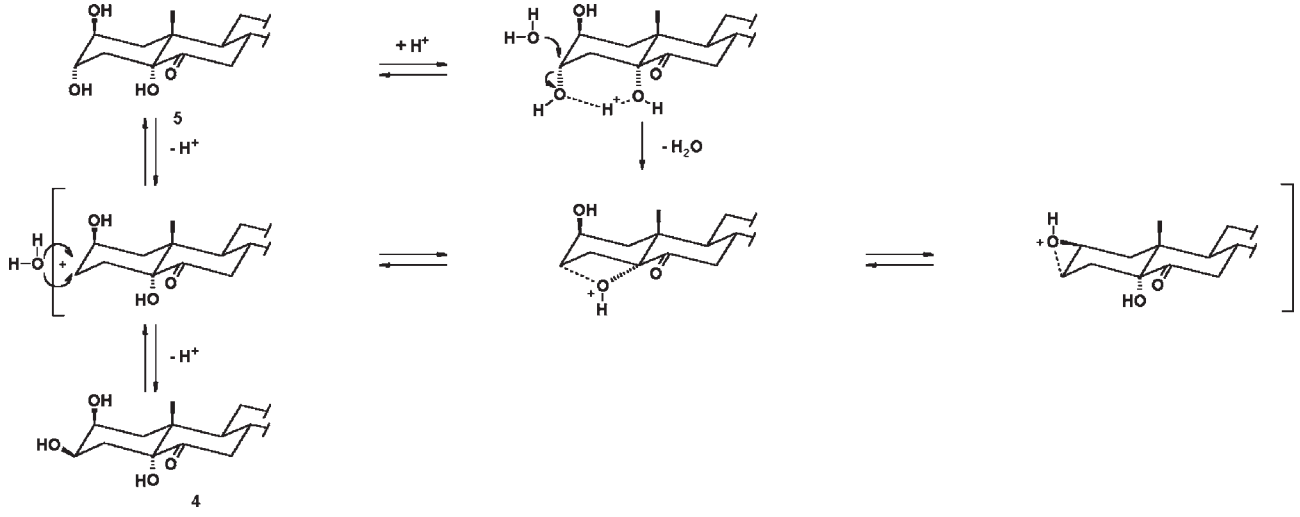
Scheme 1. Preparation of 5α-hydroxyspirostane BS analogues. Reagents and conditions: a) MCPBA/CH_2Cl_2; b) $HClO_4/H_2O/Me_2CO$; c) $HClO_4/H_2O$/MeOH.

trihydroxy compound **4** can be explained either by a S_N2 (Scheme 2) or a S_N1 (Scheme 3) mechanism, or both simultaneously. Similarly, methanolysis of the epoxide **3** in acid medium produced (25R)-2β-methoxy-3α,5α-dihydroxyspirostan-6-one (**6**) in 46% yield.

Configurations at C-2 and C-3 in β-*cis*-diol **4** were established by comparison with chemical shifts and coupling constants reported for this compound.[6] The *trans*-diol structure for compound **5** was assigned on the basis of the downfield shift of the signals for the protons 2 and 3 at δ 3.89 ppm, whereas the position of the protons 7 did not change significantly. ^{13}C NMR analysis of this compound was also consistent with the assigned structure (see Table 1). The configuration and position of the methoxy group in the compound **6** was established on the basis of a NOE-difference experiment. NMR analysis of the steroid **6**



Scheme 2. Postulated S_N2 mechanism for the formation of β-*cis* diol **4.**



Scheme 3. Postulated S_N1 mechanism for the formation of β-*cis* diol **4.**

showed that its structure is as depicted in the formula (see Experimental section).

Biological activity

The biological activity of the compounds **3, 5** and **6** was evaluated by the radish (*Raphanus sativus* L.) hypocotyl elongation and cotyledon expansion bioassay.[7] The highest growth-promoting activity was observed at 10^{-5} mg mL^{-1}, at which the radish hypocotyls treated with epoxide **3** significantly increased their length by 20.7% over untreated control. No growth-promoting activity was observed for methoxy derivate **6**, but a significant phytotoxicity was evident at 10^{-4} mg mL^{-1}. The bioactivity of compound **5** was also examined, but no activity was observed at the tested concentrations.

Experimental

General

Melting points (mp) were measured on a Electrothermal 9100 apparatus and are uncorrected. IR spectra were recorded in the region of 700–4000 cm^{-1} in KBr disks on a Phillips Analytical PU 9600 FTIR or on a Shimadzu-FTIR 8300 instrument. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ or DMSO-d_6 as solvent on a Bruker ACF-250 spectrometer at 250.13 and 62.9 MHz, respectively. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as the internal standard, and coupling constants (J) values are in Hertz (Hz). Number of directly attached protons for each carbon was established by DEPT pulse sequence. Elemental analyses were obtained on a Carlo-Erba EA-1108 Automost microanalyzer. Unless otherwise indicated, all solvents and reagents used were of commercial grade. Reactions were monitored by TLC on precoated plates with silica gel 60 G (Merck, 105554). Column chromatography was carried out on silica gel 60 (0.04-0.063 mm, Merck).

Synthesis of compounds

(25R)-2α,3α-epoxy-5α-hydroxyspirostan-6-one (**3**). Compound **2**[6] (500 mg, 0.58 mmol) in chloroform (5 mL) was treated with 3-chloroperoxybenzoic acid (200 mg, 1.16 mmol) at room temperature in darkness for 1 h. The reaction mixture was diluted with 5 mL of chloroform, washed with saturated solution of sodium bicarbonate (2 x 3 mL) and brine (2 x 5 mL). The organic phase was dried with anhydrous sodium sulfate, concentrated in vacuo and the crude product was chromatographed on silica gel with n-hexane/ethyl acetate (90:10) as eluant, affording compound **3** as a white solid in 68% yield. mp 211.2–211.7 ºC (from n-hexane/ethyl acetate). IR (KBr) ν_{max}/cm^{-1}: 3408 (ν_{OH}); 1713 ($\nu_{C=O}$); 1059 and 1245 (ν_{C-O}); 983, 920, 902 and 868 (spiroketal system). ^1H NMR ($CDCl_3$): 0.72 (6H, s, CH_3-18 and 19); 0.76 (3H, d, J 6.6 Hz, CH_3-27); 0.94 (3H, d, J 6.7 Hz, CH_3-21); 2.0 (1H, m, H-4β); 2.38 (1H, dd, J 1.9 and 16.6 Hz, H-4α); 2.61 (1H, t, J 12.1 Hz, H-7α); 3.27 (1H, m, H-2β); 3.30 (1H, t, J 10.3 Hz, H-26_{ax}); 3.43 (1H, m, H-26_{eq}); 3.56 (1H, m, H-3β); 4.36 (1H, m, H–16α). ^{13}C NMR ($CDCl_3$): see Table 1. Anal. Calcd. for $C_{27}H_{40}O_5$ C 72.94%, H 9.02%. Found C 72.89%, H 9.06%.

(25R)-2β,3β,5α-trihydroxy-spirostan-6-one (**4**) *and (25R)-2β,3α,5α-trihydroxyspirostan-6-one* (**5**). A mixture of **3** (220 mg, 0.49 mmol), acetone (11mL), water (1.4 mL) and 70% perchloric acid (0.2 mL) was stirred for 4 h at room temperature. Ethyl acetate (30 mL) was added and the organic layer was washed with brine (3 x 5 mL) and sodium bicarbonate (3 x 5 mL). The solvent was dried with anhydrous sodium sulfate, concentrated in vacuo and the crude product was chromatographed on silica gel with n-hexane/ethyl acetate (80:20) as eluant, first affording compound **4** in 7% yield and later compound **5** in 84% yield.

Compound **4** *((25R)-2β,3β,5α-trihydroxyspirostan-6-one)*. mp 199.2–199.5 ºC (from methanol). IR (KBr) ν_{max}/cm^{-1}: 3425 (ν_{OH}); 1723 ($\nu_{C=O}$); 1060 (ν_{C-O}); 980, 920, 902 and 865 (spiroketal system). ^1H NMR (DMSO-d_6): 0.77 (3H, s, CH_3-18); 0.79 (3H, d, J 6.6 Hz, CH_3-27); 0.91 (3H, s, CH_3-19); 0.97 (3H, d, J 6.7 Hz, CH_3-21); 3.34 (1H, t, J 10.2 Hz, H-26_{ax}); 3.47 (1H, m, H-26_{eq}); 3.84 (1H, m, H-2α); 4.41 (1H, m, H–16); 4.45 (1H, m, H-3α). ^{13}C NMR ($CDCl_3$): see Table 1.

Compound **5** *((25R)-2β,3α,5α-trihydroxyspirostan-6-one*). mp 261.4–262.3 ºC (from methanol). IR (KBr) ν_{max}/cm^{-1}: 3425 (ν_{OH}); 1725 ($\nu_{C=O}$); 1061 and 1143 (ν_{C-O}); 920, 902 and 865 (spiroketal system). ^1H NMR (DMSO-d_6): 0.71 (3H, s, CH_3-18); 0.74 (3H, d, J 6.6 Hz, CH_3-27); 0.91 (3H, d, J 6.7 Hz, CH_3-21); 0.93 (3H, s, CH_3-19); 2.62 (1H, t, J 12.2 Hz, H-7a); 3.34 (1H, t, J 10.2 Hz, H-26_{ax}); 3.47 (1H, m, H-26_{eq}); 3.89 (2H, m, H-2α and H-3β); 4.33 (1H, m, H–16). ^{13}C NMR ($CDCl_3$): see Table 1. Anal. Calcd. for $C_{28}H_{44}O_6$ C 70.09%, H 9.15%. Found C 70.04%, H 9.18%.

(25R)-2β-methoxy-3α,5α-dihydroxyspirostan-6-one (**6**). A magnetically stirred mixture of **3** (370 mg, 0.83 mmol) in methanol (30mL) and 70% perchloric acid (0.7 mL) was refluxed for 1 h. The reaction mixture was cooled and then sodium bicarbonate (400 mg) was added. The solvent was removed under vacuum, and the residue was diluted with water (5 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layer was dried with anhydrous sodium sulfate,

concentrated in vacuo and the crude product was chromatographed on silica gel with n-hexane/ethyl acetate (85:15) as eluant, affording compound **6** in 46% yield. mp 264.6–266.2 ºC (from chloroform/methanol). IR (KBr) ν_{max}/cm^{-1}: 3414 (ν_{OH}); 1700 ($\nu_{C=O}$); 1067 and 1152 (ν_{C-O}); 977, 898 and 865 (spiroketal system). ^{1}H NMR (DMSO-d_6): 0.68 (3H, s, CH_3-18); 0.72 (3H, d, *J* 6.5 Hz, CH_3-27); 0.85 (3H, s, CH_3-19); 0.89 (3H, d, *J* 6.6 Hz, CH_3-21); 1.67 (1H, d, J 15.4 Hz, H-4α); 2.1 (1H, dd, *J* 3.2 and 15.4 Hz, H-4β); 2.61 (3H, m, H-7α, 7β and 1α); 3.25 (3H, s, -OCH_3), 3.36 (3H, m, H-2α, H-26_{ax} and H-26_{eq}); 4.04 (1H, m, H-3β); 4.33 (1H, m, H-16). ^{13}C NMR ($CDCl_3$): see Table 1. Anal. Calcd. for $C_{28}H_{44}O_6$ C 70.55%, H 9.31%. Found C 70.59%, H 9.26%.

Table 1. ^{13}C NMR chemical shifts, δ (ppm), for compounds **3**-**6**

Carbon	**3**	**4**	**5**	**6**
C-1	33.5	37.2	33.2	26.7
C-2	51.2	74.7	70.8	79.8
C-3	54.0	85.6	70.2	68.3
C-4	26.4	39.6	27.1	29.1
C-5	79.0	91.3	80.5*	80.4
C-6	209.4	208.3	212.1	211.8
C-7	42.0	44.9	41.4	41.4
C-8	37.4	39.7	36.3	36.2
C-9	45.7	48.4	45.1	45.0
C-10	41.8	52.7	42.9	46.1
C-11	20.6	21.7	20.8	20.8
C-12	39.4	40.4	39.5	39.6
C-13	40.8	40.9	41.0	41.0
C-14	56.0	56.0	45.9	55.9[a]
C-15	31.5	31.5	31.2	31.3
C-16	80.4	80.4	80.3[a]	80.0
C-17	62.0	61.9	61.2	61.9
C-18	16.2	16.3	16.3	16.3
C-19	16.0	15.0	15.8	14.8
C-20	41.6	41.6	41.5	41.5
C-21	14.4	14.5	14.2	14.3
C-22	109.2	109.3	109.3	109.3
C-23	31.3	31.3	31.3	31.2
C-24	28.7	28.7	28.6	28.6
C-25	30.2	30.2	30.1	30.1
C-26	66.9	66.9	66.8	66.8
C-27	17.1	17.1	16.9	17.0
C-OCH_3				56.6[a]

[a]assignments can be interchanged.

Biological activity

The biological activity of compounds **3, 5** and **6** was tested by the radish (*Raphanus sativus* L.) hypocotyl elongation and cotyledon expansion bioassay.[7] A mother solution of each of the compounds to be tested was prepared in ethanol, at 10^{-1} mg mL^{-1}, and the samples diluted in water were applied in a concentration range of 10^{-4} to 10^{-7} mg mL^{-1}.

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