Synthesis and preliminary biological evaluation of two new 5α-hydroxyspirostanones

Vivian Leliebre-Lara, Francisco Coll*, Carlos S. Pérez and Deysma Coll

Center for Natural Products Studies, Faculty of Chemistry, University of Havana. Zapata y G. Vedado, 10400. Ciudad Habana, Cuba

The synthesis and plant growth promoting activity of two 5α-hydroxyspirostanones are reported.

Keywords: brassinosteroid analogues, spirostanones

Considerable effort has been devoted to study the structure–activity relationship of brassinosteroids.[1] Some new simpler brassinosteroid analogues bearing non-natural functionalities have been shown to be good candidates for agricultural applications.[2-4]

Promising results have been obtained with Spirostanes possessing A-and B-ring similarities to the brassinosteroids which showed plant growth promoting activity. Based on our studies[5-9] concerning the possible capability of the spiroketal side chain of mimicking, the brassinosteroid side chain, we have previously reported the synthesis and brassinosteroid-type activity of analogues with modified spiroketal side chains.[6]

Here we report the synthesis and biological evaluation of two new 5α-hydroxyspirostan-23-ones bearing additional oxygen functions on rings A and B. We focused our attention on the biological importance of the 23-oxo functions rather than on introducing the natural functions in the ring A, aiming to find synthetically simpler plant growth regulators.

Results and discussion

The advanced intermediate **2** was obtained from the previously reported[10] triol **1** by the standard diacetylation procedure Ac_2O/pyridine. Introduction of the carbonyl group at C-23 was accomplished by treatment with boron trifluoride diethyl etherate in acetic acid solution, and sodium nitrite addition, followed by chromatography over aluminum oxide (Brookman activity III) to give ketone **3** in 40% yield from **1**. Deprotection and subsequent Jones oxidation afforded the triol **4** and ketol **5** in 31% and 19% overall yields, respectively.

The biological activities of the spirostanones **4** and **5** were tested through two different bioassays in radish (*Raphanus sativus* L.); *i.e.* the cotyledons weight assay used for cytokinin-type activity and the hypocotyls length assay used for auxin-type activity.[11]

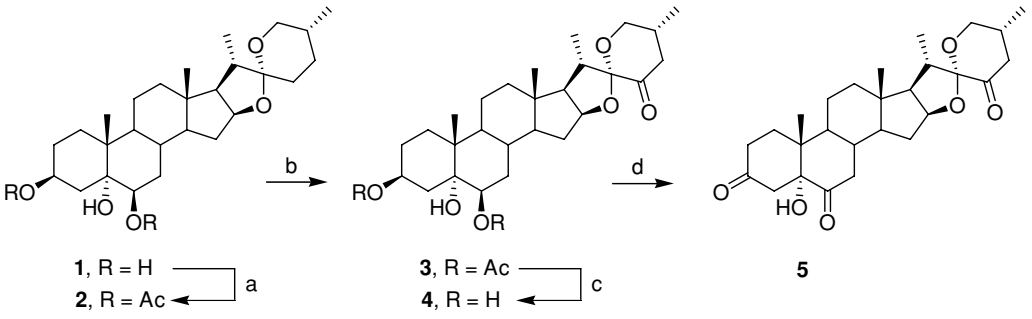
As shown in Table 2, compound **4** exhibited auxin-type activity at the concentrations 10^{-5} and 10^{-6} mg/ml; while ketol **5** displayed a moderate activity at 10^{-7} mg/ml on the same assay. Interestingly, ketol **5** showed a significant activity at the lowest concentrations 10^{-6} and 10^{-7} mg/ml in the radish cotyledons weight assay; while triol **4** showed cytokinin-type activity at the three lower concentrations, *i.e.* 10^{-4},10^{-5} and 10^{-6} mg/ml.

Table 1 ^{13}C NMR chemical shifts of compounds **2–5**

Carbon	**2**	**3**	**4**	**5**
C-1	31.3	31.5	31.6	31.8
C-2	31.6	26.6	30.5	37.2
C-3	70.8	70.7	67.1	212.0
C-4	36.5	36.6	40.3	44.5
C-5	74.6	74.7	75.0	88.6
C-6	76.0	76.0	75.9	211.0
C-7	31.8	31.8	31.9	41.8
C-8	30.3	30.3	30.4	37.8
C-9	44.9	44.9	45.1	44.6
C-10	38.5	38.5	38.4	43.1
C-11	20.8	20.7	20.8	21.2
C-12	39.9	39.7	39.7	39.2
C-13	40.6	41.2	41.2	41.5
C-14	56.5	55.7	55.7	56.1
C-15	31.5	31.6	31.6	31.5
C-16	80.7	83.2	83.2	82.9
C-17	62.0	61.7	61.7	61.7
C-18	16.3	16.3	16.3	16.0
C-19	16.6	16.4	16.4	13.9
C-20	41.6	34.8	34.7	34.7
C-21	14.4	14.1	14.3	14.3
C-22	109.2	109.8	109.8	109.8
C-23	31.3	201.9	201.9	201.9
C-24	28.7	45.2	45.2	45.2
C-25	30.3	35.8	35.8	35.8
C-26	66.8	65.6	65.6	65.7
C-27	17.1	17.0	17.0	17.0
CH_3CO	170.9	170.3	170.6	–
CH_3CO	170.3	170.8	–	–

Experimental

Melting points were determined on an Electrothermal 9100 apparatus and are uncorrected. ^1H NMR and ^{13}C NMR were recorded on a Bruker ACF-250 spectrometer at 250.13 MHz, using TMS as internal standard. Elemental analyses were performed in a Leco CHNS-932 instrument. IR spectra were recorded on KBr cell on a Philips



Scheme 1 Reagents and conditions: (a) Ac_2O/ Py; (b) *i*: AcOH/$BF_3.Et_2O$/$NaNO_2$ *ii*: Al_2O_3; (c) KOH/MeOH; (d) Jones reagent.

* Correspondent. E-mail: leliebre@fq.uh.cu

Table 2 Evaluation results of analogues **4** and **5** on the radish bioassays

Concentration (mg/ml)	Radish hypocotyls length		Radish cotyledons weight	
	4	**5**	**4**	**5**
Control	18.7 a	18.7 a	15.42 a	15.42 a
10^{-4}	20.17 ab	19.47 ab	18.09 ab	21.78 c
10^{-5}	22.4 c	20.2 ab	17.92 ab	20.05 bc
10^{-6}	22.17 bc	20.27 ab	20.43 bc	19.16 bc
10^{-7}	19.2 a	21.2 b	21.28 c	18.20 ab

Entries with similar letters do not have significant differences to $\alpha = 0.05$.

Analytical PU 9 800 FT-IR spectrometer. Duncan test was used for statistical analysis as implemented in the Statgraphic statistical program.

(25R)-5α-spirostane-3β,5,6β-triol 3,6-diacetate **(2):** 3 g (6.7 mmol) of triol **1** was dissolved in a mixture of pyridine (30 ml) and acetic anhydride (30 ml). The reaction mixture was stirred for 24 h and then poured into water. The solid was filtered, washed with 5% HCl and plenty of water, and then dried at 60°C. Recrystallisation from acetone gave 3.12 g (1.7mmol, 87%) of the diacetate **2**. m.p. (acetone): 138.6–138.8°C. IR (ν, cm^{-1}): 3436 (OH); 1736 (C=O, Ac); 1241 (C-O, Ac); 1052, 1030 (C-O); 982, 965, 899 (ν spiroketal system). ^{1}H NMR ($CDCl_3$): δ = 5.11 (1 H, br m, H-3α); 4.36 (1 H, m, H-6α); 4.38 (1 H, m, H-16); 0.80 (3 H, s, CH_3-18); 1.12 (3 H, s, CH_3-19); 0.96 (3 H; d, *J*=6.6 Hz, CH_3-21); 3.38 (1 H, dd, *J*=12.7/4.2Hz, H-26eq.); 3.45 (1 H, t, *J*=10.6 Hz, H-26ax.); 0.74 (3 H, d, *J*=6.6 Hz, CH_3-27); 1.98 (3 H, s, CH_3CO); 2.04 (3 H, s, CH_3CO). Elemental analysis: $C_{31}H_{48}O_7$, requires C 69.9%, H 9.1%; found C 69.7%, H 9.2%.

(25R)-3β,6β-diacetoxy-5α-hydroxy-5-spirostan-23-one **(3):** Sodium nitrite (1 g) was added over 1 h to a stirred solution of **2** (1g, 1.81 mmol) in acetic acid (20 ml) and boron trifluoride diethyl etherate BF_3. Et_2O (45%, 1.5 ml). After the addition was completed, the reaction mixture was stirred for an additional hour and poured into cold water. The solid was filtered, washed with water and then dissolved in the smallest amount of benzene and dried over anhydrous $MgSO_4$. The organic layer was filtered through a chromatography column of Al_2O_3 (60 g) to afford 0.53g (0.96mmol, 52%) of the ketone **3.** m.p. (hexane/EtAc): 174.0–175.9 °C. IR (ν, cm^{-1}): 3436 (OH); 1736 (C=O, Ac); 1241 (C-O, Ac); 1052, 1030 (C-O); 982, 965, 899 (ν spiroketal system). ^{1}H NMR ($CDCl_3$) δ = 5.12 (1 H, br m, H-3α); 4.71 (1 H, m, H-6α); 4.56 (1 H, m, H-16); 0.77 (3 H, s, CH_3-18); 1.14 (3 H, s, CH_3-19); 2.86 (1 H, m, H-20); 0.89 (3 H, d, *J*=7.0 Hz, CH_3-21); 3.75 (1 H, t, *J*=11.1 Hz, H-26ax); 3.55 (1 H, dd , *J*=11.2/3.8Hz, H-26eq.); 0.91 (3 H, d, *J*=6.3 Hz, CH_3-27); 1.05 (s, CH_3CO); 2.06 (s, CH_3CO). Elemental analysis: $C_{31}H_{48}O_8$, requires C 68.1%, H 8.5%; found C 67.9%, H 8.2%

(25R)- 3β,5,6β-trihydroxy-5α-spirostan-23-one **(4):** ketone **3** (299 mg, 0.54 mmol) was dissolved in 30 ml a solution of KOH/MeOH 1%. The reaction mixture was refluxed for 30 min and then poured into cold water. The precipitate was filtered, washed with water and dried at 60°C to give the compound **4** (0.2g, 77%). m.p. (acetone): 227–227 °C. IR (ν, cm^{-1}): 3430 (O-H); 1731 (C=O); 1052, 1023 (C-O) 963, 921, 862 (ν spiroketal system). ^{1}H NMR ($CDCl_3$) δ = 4.04 (1 H; br m, H-3α); 4.71 (1 H, m, H-6); 4.57 (1 H, m, H-16); 0.78 (3 H, s, CH_3-18); 1.12 (3 H, s, CH_3-19); 2.86 (1 H, t, *J*=6.8 Hz, H-20); 0.91 (3 H, d, *J*=7.9 Hz, CH_3-21); 0.92 (3 H; d, *J*=6.14 Hz, CH_3-27) Elemental analysis: $C_{27}H_{42}O_6$, requires C 70.1%, H 9.2%; found C 69.8%, H 9.3%.

(25R)-5-Hydroxy-5α-spirostan-3, 6, 23-trione **(5):** A solution of triol **4** (193.2 g, 0.42 mmol) in acetone (10 ml) was cooled to 0–5 °C and 0.2 ml of Jones reagent was added dropwise. The reaction mixture was stirred for 1 h, treated with *i*-propanol (5 ml) and poured into 50 ml of cold water. The solid was filtrated and purified by column chromatography to afford 0.4 g (0.091mmol, 61%) of compound 5. m.p. (acetone): 224–225 °C. IR (ν, cm^{-1}): 3412 (O-H); 1714, 1720 (C=O); 1033 (C-O); 965, 926, 864 (ν spiroketal system) ^{1}H NMR ($CDCl_3$): δ = 4.61 (1 H, br m, H-16); 0.77 (3 H, s, CH_3-18); 1.24 (3 H, s, CH_3-19); 2.90 (1 H, brm, H-20); 0.94 (3 H, d, *J*=6.8 Hz, CH_3-21); 3.60 (1 H, dd, *J*=11.1/4.0 Hz, H-26eq); 3.76 (1H, t, *J*=11.3Hz, H-26ax); 0.92 (3 H, d, *J*=6.8 Hz, CH_3-27). Elemental analysis: $C_{27}H_{38}O_6$, requires C 70.7%, H 8.4%; found C 70.3%, H 8.6%.

Received 15 July 2005; accepted 5 August 2005
Paper 05/3374

References

1 C. Brosa, in: *Brassinosteroids: Steroidal Plant Hormones*. A. Sakuray, T. Yokota and S. Clouse (eds) Springer-Verlag, Tokyo, 1999, pp. 199-222.
2 V.A. Khripach, V.N. Zhabinskii and A.E. de Groot, *Brassinosteroids: a New Class of Plant Hormones*, Academic Press, 1999.
3 T. Kakiushi, Y. Kamuro, S. Takatsuto and K. Kobayashi, *Agric. Biol. Chem.*, 1988, **52**, 2381.
4 S. Takatsuto, F. Futatsuya, K. Kobayashi and H. Satoh, *Agric. Biol. Chem.*, 1989, **53**, 263.
5 M.A. Iglesias-Ateaga, R. Gil, C.S. Perez and F. Coll. *J. Chem. Soc. Perkin Trans.1* 2001, **3**, 261.
6 M.A. Iglesias-Arteaga, C.S. Perez and F. Coll. *Steroids* 2002; **67**, 159.
7 C. Robaina, Y. Izquierdo, E. Alonso, F. Coll, D. Coll and M.T.A. Zullo. *J. Braz. Chem. Soc.* 2003, **14**, 466.
8 M.A. Iglesias-Ateaga, R. Gil, V. Leliebre-Lara, C.S. Perez and F. Coll. *Synth. Commun.,* 1998, **28**(8), 1381.
9 M.A. Iglesias-Ateaga, R. Gil, V. Leliebre-Lara, C.S. Perez and F. Coll. *J. Chem. Res.,* 1996, 504.
10 A.W.Z. Dawidar and Z. Naturforsch 1980, **35**, 102.
11 D.S. Letham, in: *Biochem. Physiol. Growth Subs*., F. Wightman and G. Setterfield (eds) Runge Press, Ottawa, 1968, pp.19-30.