A BRASSINOSTEROID DERIVATIVE USEFUL TO PROVIDE MORE INFORMATION ABOUT THE IMPORTANCE OF THE C_2 GROUP IN THE BRASSINOSTEROID-RECEPTOR INTERACTION

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Dedicated to the memory of Dr Václav Černý.

Synthesis and biological activity evaluation in the rice lamina inclination test of (22*R*,23*R*)-22,23-dihydroxy-5α-stigmasta-2,6-dione (**6**) and its (22*S*,23*S*)-diastereoisomer **7** is described. The activity of such compounds is discussed in terms of their ability to form hydrogen bonds by means of GRID maps. The activity elicited by **6** reinforces our idea that an oxygenated function at C_3 in a brassinosteroid is more important for biological activity than that at C_2. The results also suggest that the 2α-OH of brassinosteroids could act as an acceptor in the putative hydrogen bonding interactions in the brassinosteroid-receptor complex.
Keywords: Steroids; Brassinosteroids; Plant growth regulators; Structure–activity relationship; Phytohormones; Rice lamina inclination test; GRID map; H-Bonds.

In the field of brassinosteroids, which act as potent plant growth regulators, the identification of the minimum structural requirements needed for expressing activity is still a challenge. To achieve this goal in an efficient way, a quantitative structure–activity relationship (QSAR) has been developed in our group based on molecular modeling techniques[1]. Our results have confirmed that electrostatic charges play an important role in displaying biological activity. Moreover, based on the GRID methodology[1,2], we have found that hydrogen bonding could be one of the types of the interactions that could take place upon binding. Considering these facts, another interesting point to be determined is whether the OH groups present in a brassinosteroid act as acceptors or donors in such hydrogen bonding interactions.

In this sense, the substitution of the OH function at C_2 by a carbonyl group, and the activity evaluation of such compounds should give us more

information about the importance of an oxygenated function at C_2, and the type of interaction that could take place upon binding.

In this communication, we present the synthetic strategy towards compound **6** with only a carbonyl group at C_2 in the A-ring, and its activity evaluation in the rice lamina inclination test (RLIT). The results will be discussed in terms of the GRID methodology.

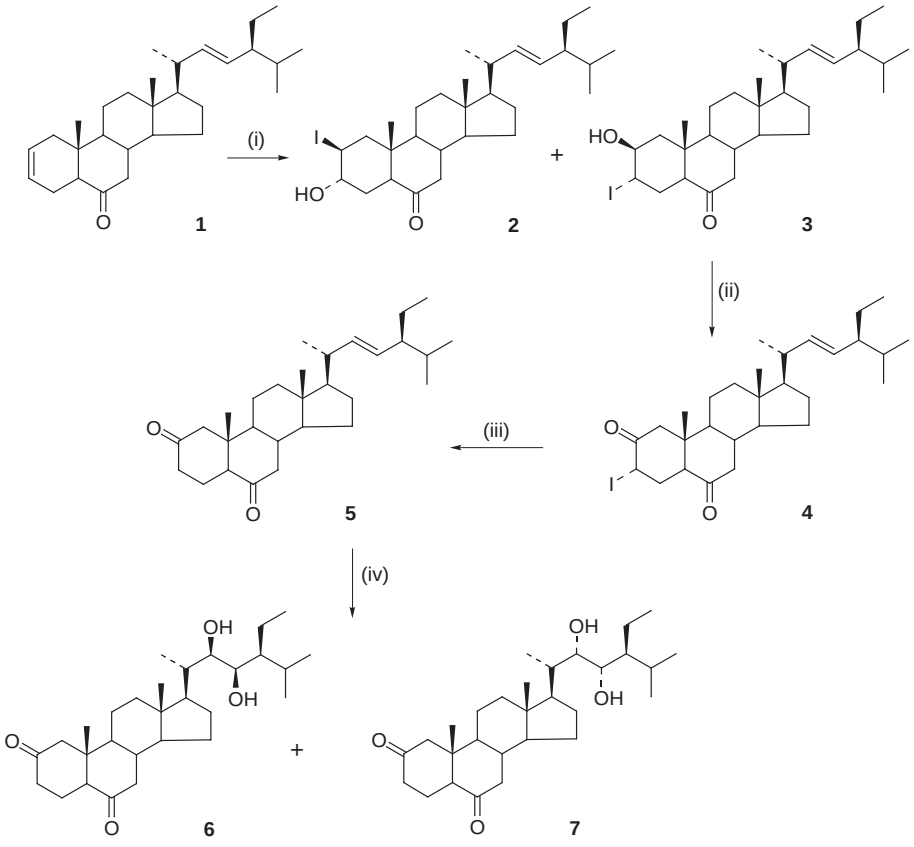
Synthesis

Retrosynthetic analysis indicated that the oxygenated function at C_2 could be introduced into the 2,3-olefine **1** by the formation of a halohydrin, which would be transformed into the desired ketone upon oxidation of the OH group and reduction of iodine.

Diene **1** was synthesized from stigmasterol in four steps following a well-known procedure[3]. Then, iodohydrin **3** was synthesized from **1** with *N*-iodosuccinimide as an electrophilic reagent[4] (see Scheme 1). Thus, using 3 equivalents of *N*-iodosuccinimide and tetrahydrofuran–water (3 : 1) as the solvent, a series of experiments was carried out by modifying both the temperature and reaction time. These results are summarised in Table I. When the reaction was carried out at –23 °C for 32 h following the procedure described by Mioskowski *et al.*[4], only **1** was recovered unchanged. In contrast, the best yield of **3** was obtained at room temperature after one hour. Prolonging the reaction time to 2.75 h afforded significant amounts of by-products **8** and **9** (see Scheme 2). Following this procedure, the two regioisomers **3** (77%) and **2** (12%) were isolated after chromatographic purification, and no compounds with iodohydrin function at C_{22}, C_{23} were detected. Subsequent oxidation of **3** with the Jones reagent

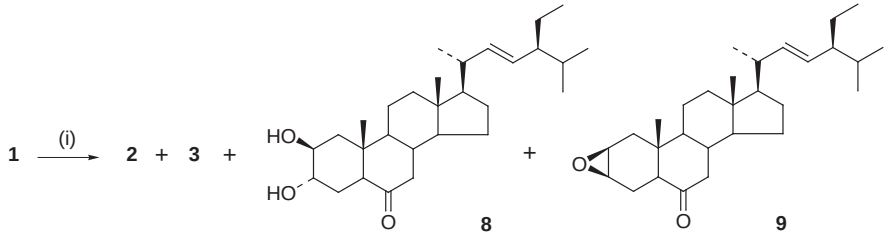
TABLE I
Effect of temperature and reaction time in the synthesis of iodohydrin **3** on yield

Entry	*T*, °C	*t*, h	Yield, %				
			1	**2**	**3**	**8**	**9**
1	–23	32	100	–	–	–	–
2	25	1	–	12	77	–	–
3	25	2.75	–	10	65–70	10–15	10



(i) NIS, THF–H_2O (3 : 1); (ii) Jones reagent; (iii) NaI, H_2SO_4 (5%);
(iv) OsO_4, *N*-methylmorpholine-*N*-oxide, H_2O, DHQD PHN

SCHEME 1



(i) NIS, THF–H_2O (3 : 1), r.t., prolonged time

SCHEME 2

(CrO_3–H_2SO_4–H_2O) to give **4**, and reductive elimination of iodine with NaI–H_2SO_4 (5%)[5] afforded **5** with an overall yield of 59% from diene **1**.

Osmium-catalyzed asymmetric dihydroxylation of the double bound of **5**, using 4-methylmorpholine-4-oxide monohydrate (NMO) as a cooxidant and dihydroquinidine 9-O-(9′-phenanthryl) ether (DHQD PHN) as a chiral ligand[6] gave, after chromatographic purification, (22*R*,23*R*)-22,23-dihydroxy-5α-stigmasta-2,6-dione (**6**) and (22*S*,23*S*)-22,23-dihydroxy-5α-stigmasta-2,6-dione (**7**) in 35 and 20% yields, respectively.

Activity Evaluation

The activity data of the new compounds were evaluated by means of a highly sensitive modified RLIT (ref.[1]), using Bahia as the cultivar, based on the procedure developed by Takeno and Pharis[7]. While **6** was shown to be active in this bioassay (–log $(dose)_{45°}$ = 1.53), **7** elicited marginal activity, at least at doses lower than 2 μg per plant. Table II shows the activity data obtained for **6** and Fig. 1 shows its dose-dependent activity curve. The activity

TABLE II
Rice lamina inclination test of **6**. Interior angle between the leaf lamina of the second leaf and its leaf sheath relative to the control

Dose, μg/plant	0.005	0.01	0.05	0.1	0.2	0.3	0.4	0.5	1.00	2.00
Angle, °	4.7	4.1	70.5	76.7	90.1	81.1	92.5	93.3	98.9	99.2

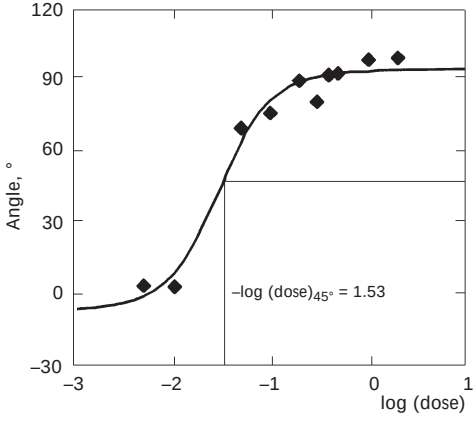


FIG. 1
Dose-dependent activity curve for **6** obtained from the activity evaluation in the rice lamina inclination test and the activity data interpolated at the angle of 45°

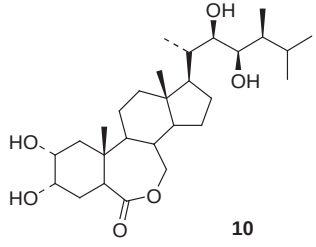
data for compounds **6** and **7** fit the prediction obtained using our QSAR model[1] (1.85 for **6** and 0.33 for **7**).

GRID Methodology

Following our own procedure[1], the GRID methodology[2] was used to calculate the interaction energy of **6** and **7** to water, used as a probe, which simulates hydrogen bonding interactions. Water was chosen due to its capability of acting both as an acceptor and donor in hydrogen bonds.

Figure 2 shows the GRID map overlap using water as the probe at –3 kcal/mol between brassinolide (**10**) (red) and **6** and **7** (yellow). The red area corresponds to the zone with higher probability of H-bonding for brassinolide (**10**) and the yellow area corresponds to the zone with higher probability of H-bonding for the brassinosteroid analogs **6** and **7**.

By comparing the GRID maps of these two brassinosteroids **6** and **7**, one can observe that the largest difference is found in the side chain, in accordance with their structural features. Thus, in the case of **6** (Fig. 2a), al-



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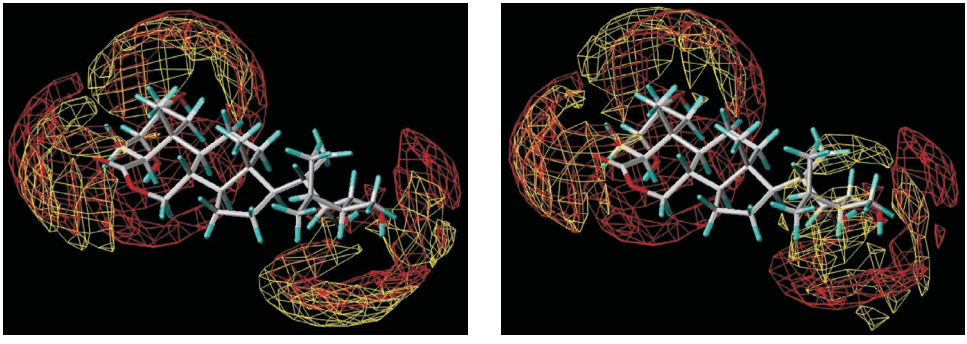


a b

FIG. 2

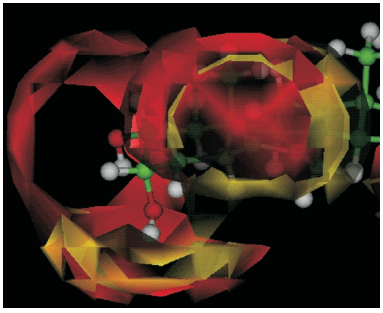
GRID map overlap for an H_2O probe at –3 kcal/mol between brassinolide (**10**) (red) and **6** (a) and **7** (b) (yellow)

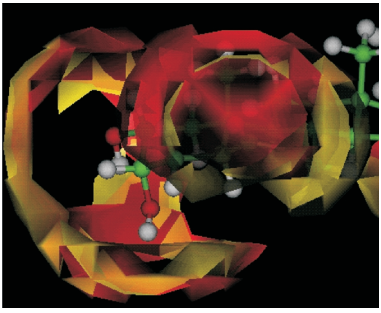
though having small differences, there is a good overlap between the GRID maps of **6** and **10** mainly in the area corresponding to the oxygenated function at C_2. On the contrary, a significant difference is observed in the overlapped maps of **7** and **10**, especially in the areas corresponding to the side chain (Fig. 2b). This is in full agreement with the results obtained when the GRID map of **10** is overlapped with other brassinosteroid analogs having 22*S*,23*S*-diol in the side chain, which is linked to a decrease in the activity[1]. Moreover, a shift to the left is observed in the area corresponding to the C_2-carbonyl of **7**.

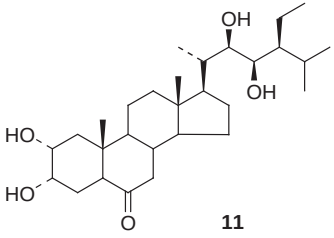
Discussion

The results obtained for **6** are discussed by comparing them with those of the previously described brassinosteroid analogs[1] **11**, **12**, and **13**. All of them differ only in the functionalities present in the A-ring. By comparing the GRID maps of these four brassinosteroids, one can observe that the largest difference is found in the A-ring, in accordance with their structural features. To see better the spatial location of these areas, Fig. 3 represents only the GRID map of the A- and B-rings of brassinolide (**10**) (red) overlapped with the same region of the other compounds, **6**, **11**, **12**, and **13** (yellow). The stick and ball representation of brassinolide (**10**), which appears in all the pictures, is also taken as a reference. Qualitatively, there is a close relationship between the area with a high probability to form an H-bond and the activity. 28-Homocastasterone (**11**) is the compound with the highest activity (2.80) of the compounds shown in Fig. 3, and the substance has a 2α,3α-diol. The activity progressively decreases in the order of **11**, **12**, **6**, and **13**. Thus, when only the 3α-OH is present (compound **12**) the activity decreases from 2.80 to 1.91. When only a 2-ketone is present (compound **6**) the activity further falls down to 1.53. Finally, the activity of ketone **13**, with no kind of functionality in the A-ring, equals to 1.00. These results are totally in agreement with the fact that the presence of a 2α,3α-diol is essential in order for a compound to possess a high activity, but they also reinforce our idea that an oxygenated function at C_3 is more important than that at C_2 (ref.[1]). Moreover, the activity displayed by **6** suggests that the 2α-OH group present in the most active brassinosteroid analogs could act as an acceptor in the putative hydrogen bonding interactions in the brassinosteroid-receptor complex.

In the case of **7**, one should take into account that, in general, brassinosteroids having a 22*S*,23*S*-diol at the side chain are less active than the corresponding 22*R*,23*R*-diastereoisomers. This can be explained by considering







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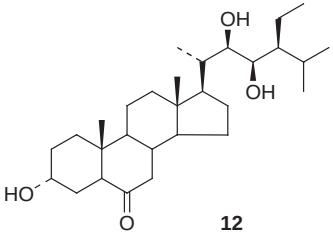

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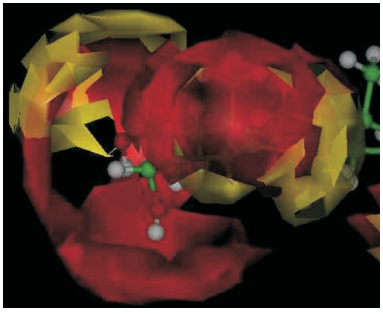
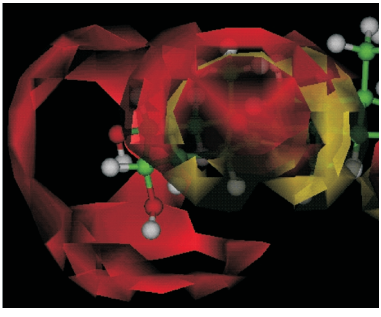
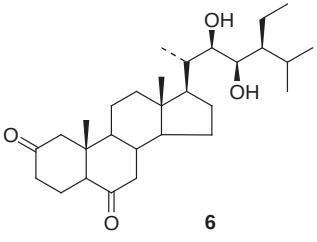
Activity (–log (dose)$_{45°}$): 2.80 1.91







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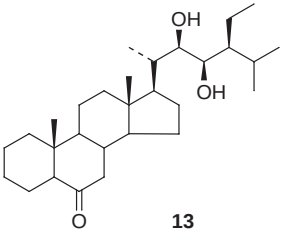

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Activity (–log (dose)$_{45°}$): 1.53 1.00

FIG. 3

A- and B-ring GRID maps overlap for an H_2O probe at –3 kcal/mol between brassinolide (**10**) (red) and **6**, **11**, **12**, **13** (yellow). Activity (–log (dose)$_{45°}$) in the rice lamina inclination test

the bad GRID map overlap between the 22*S*,23*S*-side chain analogs and brassinolide (**10**). An example of this is the activity elicited by (22*S*,23*S*)-28-homocastasterone (1.14) compared to that of 28-homocastasterone (**11**) (2.80). Therefore, bad overlap between the side chains of **7** and **10** (Fig. 2b) and the absence of an oxygenated function at C_3 can explain the marginal activity of **7**.

EXPERIMENTAL

Rice Lamina Inclination Test (RLIT)

Seeds of the Bahia rice cultivar were soaked in water and incubated in a growth chamber under a 16 h light/8 h darkness photoperiod at 30 °C for two days. The germinated seeds were planted on the surface of 0.5% aqueous agar medium and incubated under the above conditions for four days. Selected seedlings were treated with the brassinosteroid test solutions (95% ethanol) by applying them with a microsyringe (0.5 μl) onto the second lamina joint of the plant sheath. Treated and untreated (control) seedlings were returned to the growth chamber at 30 °C in the dark for two days. The interior angle between the leaf lamina of the second leaf and its leaf sheath was then measured and statistical parameters calculated.

GRID Methodology

GRID map calculations were performed on the active conformations[1] of **6** and **7** using the GRID and GOLPE programs[8].

Synthesis

Melting points were determined on a Büchi 530 instrument and are uncorrected. IR spectra of chloroform solutions were obtained on a Perkin–Elmer 683 spectrometer. Wavenumbers are given in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer using deuteriochloroform with TMS as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. The multiplicity of the signals in the ^{13}C NMR spectra was determined using the DEPT sequence. Mass spectra of positive ions obtained by chemical ionization (CI, 70 eV) were mesured on a Hewlett–Packard 5988-A instrument using methane as the carrier gas. High resolution mass spectra (*m/z* (h.r.)) were measured on a Fisions VG-Altospec spectrometer. The progress of all reactions and column chromatography was monitored by TLC on silica gel $60F_{254}$ microplates (Macherey–Nagel (MN), Art 804023) and the spots were detected by spraying with 50% sulfuric acid, followed by heating. Flash column chromatography was performed on 230–400 mesh, MN silica gel. Column chromatography was performed on MN silica gel (70–230 mesh).

(22*E*)-2β-Hydroxy-3α-iodo-5α-stigmast-22-en-6-one (**3**)

A solution of (22*E*)-5α-stigmasta-2,22-dien-6-one (**1**) (2.00 g, 4.87 mmol) and *N*-iodosuccinimide (NIS) (3.33 g, 14.8 mmol) in THF–H_2O (3 : 1, 64 ml) was stirred at room temperature in the dark for 1 h. The solvent was evaporated to dryness and the mixture was dissolved in diisopropyl ether. The organic phase was washed with brine and water, dried over anhy-

drous $MgSO_4$, and evaporated to dryness. The mixture was chromatographed by flash column chromatography (Cy–AcOEt 7 : 1) to give 2.072 g (77%) of **3** and 321 mg (12%) of (22*E*)-3α-hydroxy-2β-iodo-5α-stigmast-22-en-6-one (**2**).

(22E)-2β-Hydroxy-3α-iodo-5α-stigmast-22-en-6-one (**3**): m.p. 147–149 °C (decomp.). IR: 3 456; 2 955; 2 869; 1 703; 1 460; 1 381; 1 009; 974; 758. CI MS, *m/z* (rel.%): 555 (94) $[M + 1]^+$, 537 (7) $[(M + 1) - H_2O]^+$, 511 (32), 442 (54), 427 (100) $[(M + 1) - HI]^+$, 413 (52), 383 (21), 314 (28), 287 (41). CI MS, *m/z* (h.r.): 555.2668 $[M + 1]^+$ (calculated 555.2699). ^{1}H NMR: 0.69 s, 3 H (3 × H-18); 0.80 d, 3 H, *J*(25,27) = 6.6 (3 × H-27); 0.81 t, 3 H, *J*(28,29) = 7.2 (3 × H-29); 0.85 d, 3 H, *J*(25,26) = 6.6 (3 × H-26); 0.98 s, 3 H (3 × H-19); 1.03 d, 3 H, *J*(20,21) = 6.6 (3 × H-21); 4.33 m, 1 H (H-2α); 4.55 m, 1 H (H-3β); 5.02 dd, 1 H, *J*(22,23) = 15.0, *J*(23,24) = 8.4 (H-23); 5.15 dd, 1 H, *J*(22,23) = 15.0, *J*(20,22) = 8.4 (H-22). ^{13}C NMR: 12.2 q (C-18); 12.2 q (C-29); 16.0 q (C-19); 19.0 q (C-27); 21.1 q (C-26); 21.1 t (C-11); 21.2 q (C-21); 24.0 t (C-15); 24.9 t (C-4); 25.4 t (C-28); 28.7 t (C-16); 31.9 d (C-25); 34.7 d (C-3); 37.2 d (C-8); 38.0 t (C-1); 39.3 t (C-12); 40.5 d (C-20); 41.3 s (C-10); 42.9 s (C-13); 46.5 t (C-7); 51.2 d (C-24); 54.9 d, 54.4 d (C-5, C-9); 55.9 d (C-17); 56.7 d (C-14); 72.5 d (C-2); 129.6 d (C-23); 138.0 d (C-22); 211.3 s (C-6).

(22E)-3α-Hydroxy-2β-iodo-5α-stigmast-22-en-6-one (**2**): m.p. 134–136 °C (decomp.). IR: 3 430; 2 955; 2 869; 1 701; 1 460; 1 381; 1 009; 974; 758. CI MS, *m/z* (rel.%): 555 (34) $[M + 1]^+$, 537 (49) $[(M + 1) - H_2O]^+$, 493 (23), 442 (27), 427 (100) $[(M + 1) - HI]^+$, 381 (30), 330 (42), 314 (30), 293 (42), 281 (68). CI MS, *m/z* (h.r.): 555.2696 $[M + 1]^+$ (calculated 555.2699). ^{1}H NMR: 0.68 s, 3 H (3 × H-18); 0.79 d, 3 H, *J*(25,27) = 6.6 (3 × H-27); 0.80 t, 3 H, *J*(28,29) = 7.2 (3 × H-29); 0.84 d, 3 H, *J*(25,26) = 6.6 (3 × H-26); 1.02 d, 3 H, *J*(20,21) = 6.6 (3 × H-21); 1.11 s, 3 H (3 × H-19); 4.40 m, 1 H (H-3β); 4.46 m, 1 H (H-2α); 5.02 dd, 1 H, *J*(22,23) = 15.0, *J*(23,24) = 8.4 (H-23); 5.15 dd, 1 H, *J*(22,23) = 15.0, *J*(20,22) = 8.4 (H-22). ^{13}C NMR: 12.2 q (C-18); 12.2 q (C-29); 15.4 q (C-19); 19.0 q (C-27); 21.1 q (C-26); 21.1 q (C-21); 22.8 t (C-4); 24.0 t (C-15); 25.4 t (C-28); 26.7 d (C-2); 28.7 t (C-16); 31.8 d (C-25); 37.1 d (C-8); 39.4 t (C-12); 40.4 d (C-20); 40.5 t (C-1); 41.8 s (C-10); 42.9 s (C-13); 46.5 t (C-7); 51.2 d (C-24); 50.7 d, 55.3 d (C-5, C-9); 55.8 d (C-17); 56.7 d (C-14); 72.4 d (C-3); 129.6 d (C-23); 137.9 d (C-22); 211.7 s (C-6).

(22*E*)-3α-Iodo-5α-stigmast-22-ene-2,6-dione (**4**)

Jones reagent (approx. 0.1 ml) was added dropwise to a stirred solution of (22*E*)-2β-hydroxy-3α-iodo-5α-stigmast-22-en-6-one (**3**) (100 mg, 0.18 mmol) in acetone (1.6 ml). The mixture was stirred at 0 °C for 15 min, and isopropyl alcohol was subsequently added to quench the reaction. The mixture was filtered over Celite and the solvent was evaporated to dryness. The crude material was chromatographed (Cy–AcOEt 9 : 1) to give **4** (85 mg, 85%). M.p. 130–131 °C (decomp.). IR: 2 957; 2 868; 1 710. CI MS, *m/z* (rel.%): 553 (100) $[M + 1]^+$, 427 (85), 411 (31), 383 (27), 314 (31), 285 (42). CI MS, *m/z* (h.r.): 553.2531 $[M + 1]^+$ (calculated 553.2543). ^{1}H NMR: 0.68 s, 3 H (3 × H-18); 0.68 s, 3 H (3 × H-19); 0.80 d, 3 H, *J*(25,27) = 6.6 (3 × H-27); 0.81 t, 3 H, *J*(28,29) = 7.2 (3 × H-29); 0.85 d, 3 H, *J*(25,26) = 6.6 (3 × H-26); 1.03 d, 3 H, *J*(20,21) = 6.6 (3 × H-21); 4.67 m, 1 H (H-3β); 5.02 dd, 1 H, *J*(22,23) = 15.0, *J*(23,24) = 8.4 (H-23); 5.15 dd, 1 H, *J*(22,23) = 15.0, *J*(20,22) = 8.4 (H-22). ^{13}C NMR: 12.1 q (C-18); 12.2 q (C-29); 14.4 q (C-19); 19.0 q (C-27); 21.0 t (C-11); 21.1 q (C-26); 21.2 q (C-21); 24.0 t (C-15); 25.4 t (C-28); 28.5 d (C-3); 28.7 t (C-16); 30.9 t (C-4); 31.8 d (C-25); 37.6 d (C-8); 39.0 t (C-12); 40.4 d (C-20); 42.7 s (C-13); 45.3 s (C-10); 46.4 t, 46.6 t (C-1,

C-7); 51.2 d (C-24); 52.9 d (C-5); 52.9 d (C-9); 55.8 d (C-17); 56.7 d (C-14); 129.7 d (C-23); 137.8 d (C-22); 204.3 s, 209.3 s (C-2, C-6).

(22*E*)-5α-Stigmast-22-ene-2,6-dione (**5**)

A solution of **4** (290 mg, 0.68 mmol), NaI (419 mg, 2.79 mmol) and H_2SO_4 (0.3 ml, 5%) in THF–H_2O (1 : 1, 6 ml) was stirred at room temperature for 16 h. A solution of $NaHCO_3$ was then added to quench the reaction. The mixture was extracted with AcOEt, and the organic phase was washed with saturated solution of $NaHCO_3$ and water, dried over anhydrous $MgSO_4$, and evaporated to dryness. The crude material was chromatographed ($CHCl_3$, AcOEt) to give **5** (201 mg, 90%). M.p. 185–186 °C. IR: 2 955; 2 935; 2 902; 2 868; 1 748; 1 707; 1 460; 1 380; 1 368; 1 326; 973. CI MS, *m/z* (rel.%): 427 (100) $[M + 1]^+$, 383 (32) $[M - C_3H_8]^+$, 314 (37), 285 (44). CI MS, *m/z* (h.r.): 427.3582 $[M + 1]^+$ (calculated 427.3576). ^{1}H NMR: 0.68 s, 3 H (3 × H-18); 0.72 s, 3 H (3 × H-19); 0.79 d, 3 H, *J*(25,27) = 6.6 (3 × H-27); 0.80 t, 3 H, *J*(28,29) = 7.2 (3 × H-29); 0.85 d, 3 H, *J*(25,26) = 6.6 (3 × H-26); 1.02 d, 3 H, *J*(20,21) = 6.6 (3 × H-21); 5.02 dd, 1 H, *J*(22,23) = 15.0, *J*(23,24) = 8.4 (H-23); 5.15 dd, 1 H, *J*(22,23) = 15.0, *J*(20,22) = 8.4 (H-22). ^{13}C NMR: 12.1 q (C-18); 12.2 q (C-29); 14.2 q (C-19); 19.0 q (C-27); 21.0 q (C-26); 21.1 q (C-21); 21.1, 21.3 (C-4, C-11); 24.0 t (C-15); 25.4 t (C-28); 28.7 t (C-16); 31.8 d (C-25); 37.8 d (C-8); 39.1 t (C-12); 40.0 t, 53.3 t (C-1, C-3); 40.4 d (C-20); 42.7 s (C-13); 45.5 s (C-10); 46.7 t (C-7); 51.2 d (C-24); 53.9 d (C-9); 55.8 d (C-17); 56.8 d (C-5); 56.8 d (C-14); 129.7 d (C-23); 137.8 d (C-22); 210.1 s, 210.3 s (C-2, C-6).

(22*R*,23*R*)-22,23-Dihydroxy-5α-stigmasta-2,6-dione (**6**) and
(22*S*,23*S*)-22,23-dihydroxy-5α-stigmasta-2,6-dione (**7**)

A solution of **5** (97 mg, 0.23 mmol) in THF (2 ml) was slowly added dropwise to a solution of OsO_4 (11 mg, 0.04 mmol), dihydroquinidine 9-*O*-(9′-phenanthryl) ether (0.24 g, 0.48 mmol), *N*-methylmorpholine-*N*-oxide (1.9 g, 14.1 mmol) and $Et_4N^+AcO^-\cdot nH_2O$ (0.5 g, 1.88 mmol) in THF (4 ml), *t*-ButOH (4.3 ml) and H_2O (1 ml). The mixture was stirred under argon atmosphere at 0 °C in the dark for 10 days. The reaction mixture was treated with a saturated solution of $Na_2S_2O_5$ (10 ml) and stirred at room temperature for 1 h. The mixture was extracted with $CHCl_3$, the organic phase was washed with 0.5 M H_2SO_4, saturated solutions of $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. Removal of the solvent yielded 184 mg of crude product, which was purified by chromatography (Cy–AcOEt 3 : 1) to give **6** (36 mg, 35%) and **7** (21 mg, 20%).

(22R,23R)-22,23-Dihydroxy-5α-stigmasta-2,6-dione (**6**): m.p. 180–182 °C. IR: 3 465; 2 956; 2 871; 1 711; 1 462; 1 382; 1 232; 980; 756. CI MS, *m/z* (rel.%): 461 (37) $[M + 1]^+$, 346 (100) $[M - C_7H_{16}O]^+$, 327 (23), 287 (21), 219 (22), 181 (35). CI MS, *m/z* (h.r.): 461.3643 $[M + 1]^+$ (calculated 461.3631). ^{1}H NMR: 0.68 s, 3 H (3 × H-18); 0.72 s, 3 H (3 × H-19); 0.91 d, 3 H, *J*(20,21) = 6.3 (3 × H-21); 0.95 t, 3 H, *J*(28,29) = 7.5 (3 × H-29); 0.96 d, 3 H, *J* = 6.9 and 0.97 d, 3 H, *J* = 6.9 (3 × H-26, 3 × H-27); 3.59 dd, 1 H, *J* = 8.7, *J*′ = 1.5 (H-22); 3.73 dd, 1 H, *J* = 8.7, *J*′ = 1.5 (H-23). ^{13}C NMR: 11.8 q (C-18); 11.9 q (C-21); 13.5 q (C-29); 14.2 q (C-19); 18.8 t (C-28); 19.4 q (C-26); 21.2 q (C-27); 21.2, 21.3 (C-4, C-11); 23.8 t (C-15); 27.6 t (C-16); 28.9 d (C-25); 36.9 d (C-20); 37.8 d (C-8); 39.3 t (C-12); 40.0 t, 53.3 t (C-1, C-3); 42.7 s (C-13); 45.5 s (C-10); 46.4 d (C-24); 46.7 t (C-7); 52.5 d (C-17); 53.4 d (C-9); 56.5 d (C-14); 56.8 d (C-5); 72.8 d (C-23); 74.5 d (C-22); 210.0 s, 210.2 s (C-2, C-6).

(22S,23S)-22,23-Dihydroxy-5α-stigmasta-2,6-dione (**7**): m.p. 188–190 °C. IR: 3 454; 2 957; 2 928; 2 870; 2 854; 1 739; 1 713; 1 463; 1 380; 1 260; 1 095; 1 039; 1 023; 804. CI MS, *m/z* (rel.%): 461 (21) $[M + 1]^+$, 346 (100) $[M - C_7H_{16}O]^+$, 316 (36), 287 (21), 219 (26), 181 (40), 169 (53). CI MS, *m/z* (h.r.): 461.3644 $[M + 1]^+$ (calculated 461.3631). ^{1}H NMR: 0.71 s, 3 H (3 × H-18); 0.72 s, 3 H (3 × H-19); 0.88 d, 3 H, *J* = 6.9 and 0.95 d, 3 H, *J* = 6.9 (3 × H-26, 3 × H-27); 0.96 t, 3 H, *J*(28,29) = 7.5 (3 × H-29); 1.04 d, 3 H, *J*(20,21) = 6.9 (3 × H-21); 3.57–3.64 m, 2 H (H-22, H-23). ^{13}C NMR: 11.8 q (C-18); 14.1 q (C-21); 14.2 q (C-19); 14.5 q (C-29); 17.6 q (C-27); 18.5 t (C-28); 21.1, 21.3 (C-4, C-11); 21.7 q (C-26); 24.2 t (C-15); 26.8 d (C-25); 27.7 t (C-16); 37.7 d (C-8); 39.1 t (C-12); 39.9 t, 53.2 t (C-1, C-3); 42.2 d (C-20); 43.3 s (C-13); 45.5 s (C-10); 46.6 t (C-7); 49.5 d (C-24); 52.5 d (C-17); 53.3 d (C-9); 56.2 d (C-14); 56.8 d (C-5); 70.6 d (C-23); 72.1 d (C-22); 210.0 s, 210.3 s (C-2, C-6).

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