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Partial Synthesis of Nitrogenous Brassinosteroid Analogues with Solanidane Skeleton[1]

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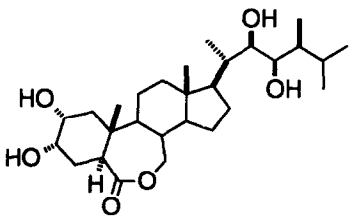
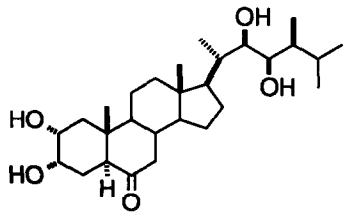
Abstract: Starting with the *Solanum* steroid alkaloid solanidine (**3**), the nitrogenous brassinosteroid analogues 2α,3α-dihydroxy-5α,22α*H*,25ß*H*-solanidan-6-one (**10**) and 2α,3α-dihydroxy-6,7-seco-5α,22α*H*,25ß*H*-solanidano-6,7-lactone (**15**) as well as some of their derivatives (e. g., the **10** and **15** *N*-oxides **12** and **16**) and the 5,6-seco-6,5-isolactone **18** have been synthesized.

The brassinosteroids represent a new class of steroidal phytohormones of an ubiquitous distribution in the plant kingdom with high growth promoting and antistress activities[3-5]. Since the discovery of brassinolide (**1**) in 1979[6], extensive chemical research work has been done directed to the isolation and structural elucidation of new naturally occurring brassinosteroids as well as to the synthesis of these compounds as basis for further biological investigations. Thus, up to now more than thirty endogenous brassinosteroids have been characterized, among them brassinolide (**1**) and its biogenetic precursor castasterone (**2**), which are considered to be the most important members due to their wide distribution and potent biological activity[7]. As part of a programme directed toward the synthesis of analogues with brassinosteroidal or antibrassinosteroidal activities, in addition to brassinosteroids with a spirostan side chain moiety[8], we were interested in nitrogenous brassinosteroid analogues with solanidane, spirosolane, and 22,26-epiminocholestane skeleton having the typical functionalization of **1** and **2** in rings A/B. In this publication we report the synthesis of the solanidane-derived compounds 2α,3α-dihydroxy-5α,22α*H*,25ß*H*-solanidan-6-one (**10**) and 2α,3α-dihydroxy-6,7-seco-5α,22α*H*,25ß*H*-solanidano-6,7-lactone (**15**) as well as some of their derivatives from the *Solanum* steroid alkaloid solanidine (**3**).

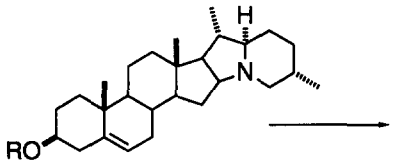


1 (Brassinolide)

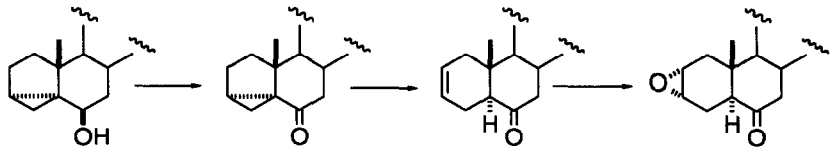


2 (Castasterone)

Solanidine (22α*H*,25ß*H*-solanid-5-en-3ß-ol, **3**)[9,10] was converted into its known *O*-tosyl derivative **4**[11] which, by heating with potassium acetate in acetone, afforded the new 5α,5-cyclo-5α,22α*H*,25ß*H*-solanidan-6ß-ol (**5**) in 91 % yield. Subsequent oxidation with chromium(VI) oxide in pyridine (70 % of **6**) and isomerization with pyridine hydrobromide in DMF at 153 °C yielded 88 % of 5α,22α*H*,25ß*H*-solanid-2-en-6-one (**7**).



3: R = H (Solanidine)
4: R = Tosyl



5 **6** **7** **9**
8: *N*-oxide

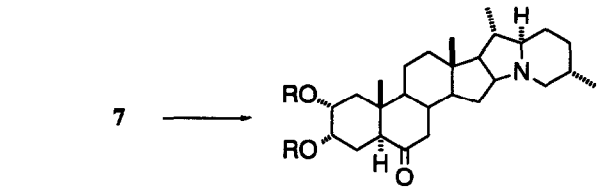
Oxidation of compound **7** with one mole of *m*-chloroperoxyperbenzoic acid (MCPBA) in dichloromethane at room temperature for 45 min gave in 77 % yield the respective *N*-oxide **8**. Its EI mass spectrum shows in addition to the typical fragmentation pattern of the solanidanes[10] the prominent fragment ions at *m/z* 394 [M^+ – OH], 342 [$M^+ - C_5H_9$], and 114 [$C_6H_{12}NO^+$], characteristic of solanidane *N*-oxides[12]. However, treatment of **7** with five moles of MCPBA for 24 hs, followed by reduction of the obtained *N*-oxide moiety with $NaHSO_3$ afforded 2α,3α-epoxy-5α,22α*H*,25ß*H*-solanidan-6-one (**9**) in 62 % yield.

To prepare the castasterone analogue **10** with solanidane skeleton, compound **7** was hydroxylated with osmium(VIII) oxide/*N*-methylmorpholine *N*-oxide in THF for 10 hs. Chromatography on silica gel gave in 55 % yield the desired 2α,3α-dihydroxy-5α,22α*H*,25ß*H*-solanidan-6-one (**10**). Acetylation yielded the 2,3-*O*-diacetate **11**. Treatment of the compounds **10** and **11** with MCPBA in dichloromethane afforded the corresponding *N*-oxides **12** and **13**, respectively, the EI mass spectra of which showing also the characteristic fragment ions of the solanidane *N*-oxide moiety[12].

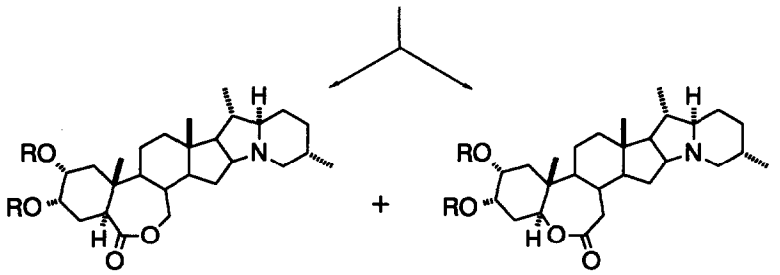
Baeyer-Villiger oxidation of 2α,3α-diacetoxy-5α,22α*H*,25ß*H*-solanidan-6-one (**11**) with trifluoroperoxyacetic acid analogously to Ikekawa *et al.*[13] afforded a mixture of two main compounds which was separated by flash chromatography on silica gel. According to their ^{1}H-NMR and mass spectra (see the Experimental Section), the major compound (43 %) was shown to be identical with 2α,3α-diacetoxy-6,7-seco-5α,22α*H*,25ß*H*-solanidano-6,7-lactone (**14**), the minor compound (19 %) with the respective 5,6-seco-6,5-isolactone diacetate **17**. Hydrolysis of **14** and **17** yielded the requested 2α,3α-dihydroxy-6,7-seco-5α,22α*H*,25ß*H*-solanidano-6,7-lactone (**15**) as well as the 5,6-seco-6,5-isolactone **18**. Both compounds

15 and **18** are also obtained by direct Baeyer-Villiger oxidation of the non-acetylated 2α,3α-dihydroxy-5α,22α*H*,25ß*H*-solanidan-6-one (**10**) in distinct higher yields (52 % and 25 %, respectively). Treatment of the compound **15** with MCPBA in dichloromethane yielded the corresponding *N*-oxide **16**.

The structures of the hitherto not described compounds **5-18** were in accordance with the corresponding NMR, MS, and further spectral data (see the Experimental Section).



10: R = H
11: R = Ac
12: R = H, *N*-oxide
13: R = Ac, *N*-oxide



14: R = Ac
15: R = H
16: R = H, *N*-oxide

17: R = Ac
18: R = H

For the determination of the brassinosteroidal or antibrassinosteroidal activity of the nitrogenous brassinosteroid analogues **10**, **12**, **15**, and **16**, the highly sensitive and specific rice lamina inclination test[14] has been used with 24-epibrassinolide [(24*R*)-**1**] as standard. By application of 0.1 ppm of the compounds, only the brassinolide analogue **15** showed a low activity of about 10-15 % compared to 24-epibrassinolide. The castasterone analogue **10** as well as the *N*-oxides **12** and **16** were practically inactive or, in the case of the *N*-oxides, even slightly toxic in this bioassay. To determine a possible antibrassinosteroidal activity, 1:1 mixtures of the compounds **10** or **12** with 24-epicastasterone [(24*R*)-**2**] and **15** or **16** with 24-epibrassinolide [(24*R*)-**1**], respectively, have been tested also in each 0.1 ppm concentrations in the rice lamina inclination bioassay. According to this, no any antibrassinosteroidal activities have been observed.

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EXPERIMENTAL SECTION

General

Melting points (corrected): Micro melting point apparatus PHMK-05 (Wägetechnik Rapido) with digital thermometer DTM 2110 (Thermometerwerk Geraberg). – Optical Rotations: Zeiss Polamat A, solvent chloroform. – IR spectra: Zeiss Specord 75 IR. – UV spectra: Zeiss Specord UV-VIS, solvent methanol. – ORD: Jasco Optical Rotatory Dispersion Recorder ORD/UV-5, solvent methanol. – ^{1}H-NMR spectra: (500, 300, or 200 MHz, solvent $CDCl_3$, TMS as internal standard): Varian Unity 500, Bruker NMR Spectrometer AM 300 or WP 200 SY. – Mass spectra (70 eV, ion source temperature 200-220 °C): Double focussing mass spectrometer AMD 402. – Column chromatography: Silica gel 60, 0.040-0.063 mm (Merck), if not indicated otherwise. – Analytical and preparative TLC: DC-Alufolien Kieselgel 60 F_{254}(Merck) and silica gel G (Merck), respectively; eluant a: chloroform/methanol (90:10), b: chloroform/methanol (85:15), c: *n*-hexane/ethyl acetate (80:20); detection with concentrated sulfuric acid at 110 °C and UV fluorescence or with iodine vapor.

Synthesis of the Nitrogenous Castasterone Analogue 10

3ß-(p-Toluenesulfonyloxy)-22αH,25ßH-solanid-5-en-3ß-ol (4). To a solution of 10.0 g (25.1 mmol) of solanidine (22α*H*,25ß*H*-solanid-5-en-3ß-ol, **3**)[9,10] in 250 ml of absolute pyridine 60.0 g (315 mmol) of *p*-toluenesulfonyl chloride was added successively under argon and magnetic stirring at 0 °C during 1 h. After standing at room temp. for 24 hs, no any more solanidine was detectable by TLC (eluant a: R_f of **3** = 0.45, of **4** = 0.60). 1.5 l of ice/water was added and the rose-coloured precipitate filtered off, washed with water, and dried. After crystallization from pyridine/water 5.1 g (37 %) of rose-coloured needles with mp 161-162 °C and $[\alpha]_D^{22} = -30.4°$ (c = 0.042) [ref.[10]: mp 161-162 °C]. – IR (KBr): ν = 1595 cm^{-1} (tosyl). – ^{1}H NMR (200 MHz): δ = 0.82 (s, 3 H, 18-H_3), 0.83 (d, *J* = 6.1 Hz, 3 H, 27-H_3), 0.91 (d, *J* = 6.1 Hz, 3 H, 21-H_3), 0.97 (s, 3 H, 19-H_3), 2.45 (s, 3 H, γ-CH_3 of tosyl), 2.63 (m, 1 H, 16α-H), 2.84 (dd, *J* = 10.3 and 3.1 Hz, 1 H, 26$_{eq}$-H), 4.33 (m, 1 H, 3α-H), 5.3 (d, *J* = 3.9 Hz, 1 H, 6-H), 7.26 (d, *J* = 8.0 Hz, 2 H, ß-H of tosyl), 7.80 (d, *J* = 8.0 Hz, 2 H, α-H of tosyl). – EIMS: *m/z* (%) = 551 (65) [M^+], 536 (15) [$M^+ - CH_3$], 379 (45) [$C_{27}H_{41}N^+$], 204 (48) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (15), 98 (43).

The above-mentioned pyridine/water phase (1.75 l) was extracted with diethyl ether (3 x 300 ml), the ether extract washed with water and dried with anhydrous sodium sulfate. Evaporation of the solvent in vacuo yielded a residue with R_f = 0.32 (eluant a), after crystallization from acetone/water 3.5 g (35 %) of colourless needles with mp 155.5-156.2 °C, which was shown to be identical with compound **5**.

3α,5-Cyclo-5α,22αH,25ßH-solanidan-6ß-ol (5). To a solution of 4.0 g (7.25 mmol) of the solanidine tosylate **4** in 250 ml of acetone and 25 ml of water 4.0 g of potassium acetate was added and the obtained solution refluxed for 5 hs. The acetone was evaporated in vacuo with simultaneous addition of a 2 % solution of ammonia. The precipitate with R_f = 0.32 (eluant a) was crystallized from acetone/water yielding 2.63 g (91 %) of **5** as fine needles with mp 155.9-156.3 °C and $[\alpha]_D^{27} = +27.3°$ (c = 0.020). – IR (nujol): ν = 3370 cm^{-1}, 1055 (OH). – ^{1}H NMR (200 MHz): δ = 0.30 (dd, *J* = 8.0 and 4.8 Hz, 1 H, 4ß-H), 0.53 (dd, *J* = 5.0 and 4.1 Hz, 1 H, 4α-H), 0.84 (d, *J* = 6.1 Hz, 3 H, 27-H_3), 0.90 (s, 3 H, 18-H_3), 0.93 (d, *J* = 6.5 Hz, 3 H, 21-H_3), 1.06 (s, 3 H, 19-H_3), 2.65 (m, 1 H, 16α-H), 2.90 (dd, *J* = 10.3 and 3.1

Hz, 1 H, 26_{eq}-H), 3.25 (dd, J = 3.0 and 3.0 Hz, 1 H, 6α-H). – EIMS: m/z (%) = 397 (60) [M^+], 396 (28) [M^+ – H], 382 (15) [$M^+ - CH_3$], 204 (60) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (8), 98 (8).

3α,5-Cyclo-5α,22αH,25βH-solanidan-6-one (6). To a solution of 5.0 g (12.6 mmol) of compound **5** in 50 ml anhydrous pyridine 5.0 g (50.0 mmol) of chromium(VI) oxide was added at – 20 °C with stirring. The reaction mixture was stirred at room temp. for further 5 hs. Then 4 ml of methanol was added and the mixture poured into 1 l of ice/water with stirring. It was alkalized with a 25 % solution of ammonia to pH = 8 and after 14 hs extracted with ethyl acetate (4 x 1 l). The extract was concentrated in vacuo to 1 l, washed with water (2 x 1 l), dried with anhydrous sodium sulfate, and evaporated in vacuo. The residue (4.5 g, R_f = 0.58 with eluant a) was flash chromatographed on silica gel with *n*-hexane/ethyl acetate (95:5) as eluant to give after crystallization from methanol/water 3.5 g (70 %) of **6** with mp 128-130 °C and $[\alpha]_D^{22}$ = + 43.5° (c = 0.040). – IR (KBr): ν = 1680 cm^{-1} (C=O). – UV: λ_{max} (lg ε) = 280 nm (1.81). – ORD: Φ_{305} = – 3079 (trough), Φ_{290} = 0, Φ_{260} = + 1000 (peak) (a = – 40.79). – ^{1}H NMR (300 MHz): δ = 0.72 (m, 1 H, 3ß-H), 0.84 (d, J = 6.5 Hz, 3 H, 27-H_3), 0.88 (s, 3 H, 18-H_3), 0.94 (d, J = 6.5 Hz, 3 H, 21-H_3), 1.02 (s, 3 H, 19-H_3), 2.65 (m, 1 H, 16α-H), 2.85 (dd, J = 10.3 and 3.1 Hz, 1 H, 26_{eq}-H). – EIMS: m/z (%) = 395 (30) [M^+], 394 (20) [M^+ – H], 380 (10) [$M^+ - CH_3$], 204 (23) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 98 (10), 78 (32).

Further elution with *n*-hexane/ethyl acetate (70:30) in the above-mentioned flash chromatography afforded 43 mg (0.8 %) of a minor by-product, after crystallization from acetone cubes with mp 191-200 °C (dec.) and the same R_f value as compound **6**, but different colour with iodine or concentrated sulfuric acid, according to the spectral data possible identical with *6-methoxy-3α,5-cyclo-5α,22αH,25βH-solanid-6-ene*. – EIMS: m/z (%) = 409 (26) [M^+], 408 (15) [M^+ – H], 394 (10) [$M^+ - CH_3$], 380 (3) [$M^+ - CH_2 - CH_3$], 204 (20) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$].

Elution with *n*-hexane/ethyl acetate (60:40) yielded 580 mg (12 %) of the starting compound **5**.

5α,22αH,25βH-Solanid-2-en-6-one (7). To a solution of 5.6 g (14.2 mmol) of compound **6** in 70 ml of freshly distilled DMF 3.2 g of pyridine hydrobromide was added. The solution was heated under argon at 153 °C for 5.5 hs. The reaction mixture was treated with 400 ml of ice/water and then alkalized with a 25 % solution of ammonia to pH = 8. After standing 14 hs at 0 °C, the precipitate was filtered off and washed with water. Crystallization twice from methanol and acetone/water gave 4.9 g (88 %) of **7** as colourless needles with mp 249-250 °C (changing to small needles at 231 °C) and $[\alpha]_D^{22}$ = + 41.8° (c = 0.040). R_f = 0.58 (eluant a). – IR (KBr): ν = 1705 cm^{-1} (C=O), 1655 (C=C). – UV: λ_{max} (lg ε) = 285 nm (2.10). – ORD: Φ_{310} = – 527 (trough), Φ_{296} = 0, Φ_{260} = + 1813 (peak) (a = – 23.41). – ^{1}H NMR (500 MHz): δ = 0.72 (s, 3 H, 19-H_3), 0.84 (s, 3 H, 18-H_3), 0.84 (d, J = 6.6 Hz, 3 H, 27-H_3), 0.94 (d, J = 6.4 Hz, 3 H, 21-H_3), 2.2-2.4 (m, 3 H, 5-H and 7-H_2), 2.64 (m, 1 H, 16α-H), 2.84 (dd, J = 10.4 and 3.3 Hz, 1 H, 26_{eq}-H), 5.58 (m, 1 H, 2-H), 5.68 (m, 1 H, 3-H). – EIMS: m/z (%) = 395 (45) [M^+], 394 (25) [M^+ – H], 380 (13) [$M^+ - CH_3$], 204 (25) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (8), 124 (9), 98 (9).

5α,22αH,25βH-Solanid-2-en-6-one N-Oxide (8). A solution of 5 mg (0.013 mmol) of compound **7** in 0.5 ml of dichloromethane was treated with 3 mg (0.015 mmol) of 85 % *m*-chloroperoxybenzoic acid (MCPBA) in 1 ml of dichloromethane under shaking at room temp. in darkness for 45 min. Evaporation of the solvent in vacuo and chromatography on 1 g of alumina (neutral, activity III) yielded the following compounds: With ethyl acetate as eluant 0.5 mg (10 %) of the starting material **7**. Elution with ethyl acetate/methanol (96:4) 4 mg (77 %) of the *N*-oxide **8** as a colourless amorphous product with R_f = 0.32

(TLC with eluant a). – ^{1}H NMR (500 MHz): δ = 0.74 (s, 3 H, 19-H_3), 0.90 (d, *J* = 6.7 Hz, 3 H, 27-H_3), 0.98 (d, *J* = 6.7 Hz, 3 H, 21-H_3), 1.09 (s, 3 H, 18-H_3), 2.46 (dd, *J* = 11.3 and 11.3 Hz, 1 H, 26_{ax}-H), 2.60 (m, w/2 = 11 Hz, 1 H, 25ß-H), 3.32 (br d, w/2 = 13 Hz, 1 H, 26_{eq}-H), 3.71 (m, 1 H, 16α-H), 5.58 (m, 1 H, 2-H), 5.70 (m, 1 H, 3-H). – EIMS: *m/z* (%) = 411 (10) [M^+], 395 (11) [M^+ – O], 394 (21) [M^+ – OH], 380 (8) [M^+ – O – CH_3], 342 (16) [M^+ – C_5H_9], 204 (29) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 114 (32) [$C_6H_{12}NO^+$].

2α,3α-Epoxy-5α,22αH,25βH-solanidan-6-one (9). A solution of 20.0 mg (0.051 mmol) of **7** in 2 ml of dichloromethane was treated with 54 mg (0.266 mmol) of 85 % MCPBA in 2 ml of dichloromethane under the same conditions as described before, but for 24 hs. The elimination of both the *N*-oxide moiety of the obtained **9** *N*-oxide and the excess of MCPBA was accomplished by shaking with a diluted solution of sodium hydrogen sulfite for 30 min. After addition of 5 ml of chloroform, the organic phase was washed with water and a diluted solution of sodium hydrogen carbonate, dried with anhydrous sodium sulfate, and concentrated to dryness in vacuo. The residue was chromatographed on 1 g of silica gel (0.015-0.040 mm, column 22 cm, ø 0.5 cm). Elution with *n*-hexane/ethyl acetate (70:30) yielded 2 mg of a minor by-product with mp 218-224 °C (needles from acetone) and R_f= 0.55 (eluant a) and with *n*-hexane/ethyl acetate (50:50) 13 mg (62 %) of the major compound **9** with mp 231-233 °C (from ethyl acetate), $[\alpha]_D^{25} = -2.8°$ (c = 0.036), and R_f = 0.40 (eluant a). – IR (KBr): ν = 1705 cm^{-1} (C=O), 800 (epoxide). – ^{1}H NMR (500 MHz): δ = 0.71 (s, 3 H, 18-H_3), 0.89 (d, *J* = 6.7 Hz, 3 H, 27-H_3), 0.92 (s, 3 H, 19-H_3), 1.00 (d, *J* = 6.4 Hz, 3 H, 21-H_3), 2.33 (dd, *J* = 8.8 and 4.3 Hz, 1 H, 7ß-H), 2.35 (dd, *J* = 10.3 and 4.0 Hz, 1 H, 5α-H), 2.64 (m, 1 H, 16α-H), 2.84 (dd, *J* = 10.3 and 3.1 Hz, 1 H, 26_{eq}-H), 3.12 (dd, *J* = 5.7 and 4.2 Hz, 1 H, 2ß-H), 3.27 (m, w/2 = 5 Hz, 1 H, 3ß-H). – EIMS: *m/z* (%) = 411 (15) [M^+], 396 (8) [M^+ – CH_3], 204 (23) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$].

2α,3α-Dihydroxy-5α,22αH,25βH-solanidan-6-one (10). A solution of 1.2 g (3.03 mmol) of compound **7** in 60 ml of freshly distilled THF was treated with 80 mg (0.31 mmol) of osmium(VIII) oxide in 10 ml of *t*-butanol and with 1.2 g (10.2 mmol) of *N*-methylmorpholine *N*-oxide in 2 ml of water. The mixture was stirred at room temp. under argon for 10 hs. The excess of osmium(VIII) oxide was reduced with a diluted aqueous solution of sodium hydrogen sulfite. The reaction mixture was concentrated in vacuo on half of its volume, 50 ml of water added, alkalized with a 25 % solution of ammonia to pH = 8, and then extracted with chloroform (3 x 50 ml). The chloroform extract was washed with water and brine, dried with anhydrous sodium sulfate, and concentrated in vacuo. The residue (1.2 g) was flash chromatographed on 60 g of silica gel. Elution with chloroform/methanol (99:1) yielded 250 mg (21 %) of the starting material **7** and with chloroform/methanol (97:3) 710 mg (55 %) of compound **10** with R_f = 0.18 (eluant a). Needles (from acetone/water) with mp 233-237 °C and $[\alpha]_D^{26} = +3.75°$ (c = 0.050). – IR (KBr): ν = 1706 cm^{-1} (C=O), 1050 (OH). – ^{1}H NMR (500 MHz): δ = 0.77 (s, 3 H, 18-H_3), 0.82 (s, 3 H, 19-H_3), 0.83 (d, *J* = 6.8 Hz, 3 H, 27-H_3), 0.93 (d, *J* = 6.7 Hz, 3 H, 21-H_3), 2.33 (dd, *J* = 13.0 and 4.5 Hz, 1 H, 7ß-H), 2.63-2.68 (m, 2 H, 5α-H and 16α-H), 2.84 (dd, *J* = 10.3 and 3.5 Hz, 1 H, 26_{eq}-H), 3.77 (m, 1 H, 2ß-H), 4.05 (m, 1 H, 3ß-H). – EIMS: *m/z* (%) = 429 (14) [M^+], 428 (8) [M^+ – 1], 414 (6) [M^+ – CH_3], 204 (23) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (10), 98 (18). – HRMS: $C_{27}H_{43}NO_3$: Calcd. 429.3243, found 429.3238.

2α,3α-Diacetoxy-5α,22αH,25βH-solanidan-6-one (11). Compound **10** (200 mg, 0.466 mmol) was acetylated with 1.5 ml of acetic anhydride and 4.5 ml of pyridine at room temp. for 5 hs. The mixture was

poured into 50 ml of ice/water and extracted with diethyl ether (3 x 50 ml). Working up as usual and chromatography on 20 g of silica gel with *n*-hexane/ethyl acetate (60:40) as eluant afforded 190 mg (79 %) of the diacetate **11** with mp 242-245 °C (needles from acetone), $[\alpha]_D^{25} = +8.2°$ (c = 0.020), and $R_f = 0.50$ (eluant a). – IR (nujol): ν = 1732 and 1730 cm^{-1} (AcO), 1704 (C=O). – UV: λ_{max} (ln) = 270 nm (1.93). – ^1H NMR (500 MHz): δ = 0.83 (s, 3 H, 18-H_3), 0.84 (d, *J* = 6.4 Hz, 3 H, 27-H_3), 0.85 (s, 3 H, 19-H_3), 0.94 (d, *J* = 6.4 Hz, 3 H, 21-H_3), 1.99 (s, 3 H, 2α-OAc), 2.08 (s, 3 H, 3α-OAc), 2.33 (dd, *J* = 12.5 and 4.0 Hz, 1 H, 7ß-H), 2.84 (dd, *J* = 10.3 and 3.0 Hz, 1 H, 26_{eq}-H), 4.95 (m, 1 H, 2ß-H), 5.38 (m, 1 H, 3ß-H). – EIMS: *m/z* (%) = 513 (68) [M^+], 512 (30) [$M^+ - 1$], 498 (16) [$M^+ - CH_3$], 470 (5) [M^+ – Ac], 204 (65) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (12), 124 (10).

2α,3α-Dihydroxy-5α,22αH,25ßH-solanidan-6-one N-Oxide (12). Compound **12** was prepared in the same manner as described for **8** by treatment of 26 mg (0.061 mmol) of **10** in 4 ml of dichloromethane with 15 mg (0.074 mmol) of 85 % MCPBA. After crystallization from chloroform/acetone 15 mg (55 %) of **12** as colourless needles with mp 247-250 °C, $[\alpha]_D^{25} = \pm 0°$ (c = 0.026), and $R_f = 0.37$ (eluant b) and 0.10 (eluant c) [R_f of **10** = 0.37 (eluant b) and 0.30 (eluant c)]. – ^1H NMR (500 MHz): δ = 0.78 (s, 3 H, 19-H_3), 0.89 (d, *J* = 6.7 Hz, 3 H, 27-H_3), 0.97 (d, *J* = 6.7 Hz, 3 H, 21-H_3), 1.09 (s, 3 H, 18-H_3), 2.33 (dd, *J* = 12.5 and 4.0 Hz, 1H, 7ß-H), 2.37 (m, w/2 = 12 Hz, 1 H, 20α-H), 2.45 (dd, *J* = 11.6 and 11.6 Hz, 1 H, 26_{ax}-H), 2.60 (m, w/2 = 11 Hz, 1 H, 25ß-H), 2.67 (dd, *J* = 12.2 and 2.8 Hz, 1 H, 5α-H), 2.73 (m, 1 H, 22α-H), 3.31 (br d, w/2 = 10 Hz, 1 H, 26_{eq}-H), 3.70 (m, 1 H, 16α-H), 3.73 (m, w/2 = 12.1 Hz, 1 H, 2ß-H), 4.05 (m, w/2 = 5 Hz, 1 H, 3ß-H). – EIMS: *m/z* (%) = 445 (4) [M^+], 430 (7) [$M^+ - CH_3$], 429 (20) [$M^+ - O$], 428 (18) [$M^+ - OH$], 427(10) [$M^+ - H_2O$], 414 (10) [429 – CH_3], 409 (10) [427 – H_2O], 376 (8) [$M^+ - C_5H_9$], 204 (35) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 114 (5) [$C_6H_{12}NO^+$].

2α,3α-Diacetoxy-5α,22αH,25ßH-solanidan-6-one N-Oxide (13). Compound **13** was prepared in the same manner as described for **8** by treatment of 5 mg (0.0097 mmol) of **11** in 1 ml of dichloromethane with 10 mg (0.049 mmol) of 85 % MCPBA, but with a reaction time of 19 hs. 5 mg (97 %) of **13** as a colourless amorphous product with $R_f = 0.36$ (eluant a). – IR (chloroform): ν = 1739 cm^{-1} and 1724 (AcO), 1707 (C=O), 1250 and 1035 (AcO), 928 ($R_2N^+–O^-$). – UV: λ_{max} (ln) = 280 nm (2.15). – ^1H NMR (500 MHz): δ = 0.87 (s, 3 H, 19-H_3), 0.90 (d, *J* = 6.8 Hz, 3 H, 27-H_3), 0.99 (d, *J* = 6.8 Hz, 3 H, 21-H_3), 1.09 (s, 3 H, 18-H_3), 1.99 (s, 3 H, 2α-OAc), 2.08 (s, 3 H, 3α-OAc), 2.35 (m, w/2 = 10 Hz, 1 H, 20ß-H), 2.40 (dd, *J* = 11.3 and 11.3 Hz, 1 H, 26_{ax}-H), 2.60 (m, w/2 = 11 Hz, 1 H, 25ß-H), 2.70 (m, 1 H, 22α-H), 3.43 (m, w/2 = 9 Hz, 1 H, 26_{eq}-H), 3.73 (m, w/2 = 11 Hz, 1 H, 16α-H), 4.95 (m, w/2 = 12 Hz, 1 H, 2ß-H), 5.39 (m, w/2 = 5 Hz, 1 H, 3ß-H). – EIMS: *m/z* (%) = 529 (12) [M^+], 512 (12) [$M^+ - OH$], 498 (5) [$M^+ - O - CH_3$], 460 (15) [$M^+ - C_5H_9$], 204 (20) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (5), 114 (36) [$C_6H_{12}NO^+$].

Synthesis of the Nitrogenous Brassinolide Analogue 15

2α,3α-Diacetoxy-6,7-seco-5α,22αH,25ßH-solanidano-6,7-lactone (2α,3α-Diacetoxy-B-homo-7-oxa-5α,22αH,25ßH-solanidan-6-one, 14) and 2α,3α-Diacetoxy-5,6-seco-5α,22αH,25ßH-solanidano-6,5-lactone (2α,3α-Diacetoxy-B-homo-6-oxa-5α,22αH,25ßH-solanidan-7-one, 17). Analogously to ref.[13], a solution of trifluoroperoxyacetic acid, prepared by addition of 2.5 ml of trifluoroacetic anhydride to 0.5 ml of 60 % hydrogen peroxide in 11 ml of dichloromethane at 0 °C, was added dropwise to a solution of 50 mg

(0.097 mmol) of compound **11** in 3 ml of dichloromethane in the presence of 300 mg pulverized disodium hydrogen phosphate with stirring at 0 °C during 4 hs. After the addition of 2 ml of a saturated aqueous solution of sodium hydrogen sulfite and then of 10 ml of water, the mixture was extracted with chloroform (3 x 15 ml). Working up as usual and evaporation of the solvent in vacuo yielded a residue, which showed by TLC (eluant a) 2 major spots with R_f = 0.58 and 0.53. Flash chromatography on 15 g of silica gel yielded with *n*-hexane/ethyl acetate (50:50) as eluant at first 22 mg (43 %) of compound **14** with R_f = 0.58 and later 10 mg (19 %) of compound **17** with R_f = 0.53.

Compound **14**: Colourless needles (from acetone) with mp 258-262 °C and $[\alpha]_D^{25}$ = + 53.0° (c = 0.020). – ^{1}H NMR (500 MHz): δ = 0.84 (d, *J* = 6.7 Hz, 3 H, 27-H_3), 0.87 (s, 3 H, 18-H_3), 0.93 (d, *J* = 6.7 Hz, 3 H, 21-H_3), 0.99 (s, 3 H, 19-H_3), 2.00 (s, 3 H, 2α-OAc), 2.11 (s, 3 H, 3α-OAc), 2.63 (m, 1 H, 16α-H), 2.84 (dd, *J* = 10.3 and 3.3 Hz, 1 H, 26_{eq}-H), 3.00 (dd, *J* = 12.2 and 4.3 Hz, 1 H, 5α-H), 4.05 (dd, *J* = 10.5 and 9.1 Hz, 1 H, 7α-H), 4.14 (dd, *J* = 12.2 and 1.2 Hz, 1 H, 7ß-H), 4.87 (m, w/2 = 12 Hz, 1 H, 2ß-H), 5.37 (m, w/2 = 5 Hz, 1 H, 3ß-H). – EIMS: *m/z* (%) = 529 (10) [M^+], 514 (18) [$M^+ - CH_3$], 498 (18) [514 – O], 204 (60) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (12), 98 (8).

Compound **17**: Colourless needles (from acetone) with mp 270-272 °C and $[\alpha]_D^{25}$ = + 40.0° (c = 0.030). – ^{1}H NMR (500 MHz): δ = 0.84 (d, *J* = 6.7 Hz, 3 H, 27-H_3), 0.86 (s, 3 H, 18-H_3), 0.93 (d, *J* = 6.7 Hz, 3 H, 21-H_3), 1.02 (s, 3 H, 19-H_3), 2.00 (s, 3 H, 2α-OAc), 2.12 (s, 3 H, 3α-OAc), 2.46 (dd, *J* = 13.5 and 11.6 Hz, 1 H, 7α-H), 2.56-2.60 (m, 2 H, 7ß-H and 22α-H), 2.83 (dd, *J* = 10.3 and 3.0 Hz, 1 H, 26_{eq}-H), 4.47 (dd, *J* = 11.3 and 5.5 Hz, 1 H, 5α-H), 4.90 (m, w/2 = 12 Hz, 1 H, 2ß-H), 5.38 (m, w/2 = 5 Hz, 1 H, 3ß-H). – EIMS: *m/z* (%) = 529 (20) [M^+], 514 (8) [$M^+ - CH_3$], 204 (36) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 137 (32), 97 (15).

2α,3α-Dihydroxy-6,7-seco-5α,22αH,25ßH-solanidano-6,7-lactone (2α,3α-Dihydroxy-B-homo-7-oxa-5α,22αH,25ßH-solanidan-6-one, 15). The diacetate **14** (20 mg, 0.038 mmol) was refluxed with 10 ml of 5 % methanolic KOH for 2 hs. After the addition of 6 ml of 6 N HCl, the mixture was stored at room temp. for 30 min. It was alkalized with a solution of sodium hydrogen carbonate to pH = 8, extracted with chloroform (3 x 25 ml), the extract washed with water, then dried with anhydrous sodium sulfate, and concentrated in vacuo: 13 mg (77 %) of pure **15** as colourless needles (from acetone) with mp 207-209 °C, $[\alpha]_D^{25}$ = + 63.6° (c = 0.030), and R_f = 0.30 (eluant b). – ^{1}H NMR (500 MHz): δ = 0.84 (d, *J* = 6.4 Hz, 3 H, 27-H_3), 0.86 (s, 3 H, 18-H_3), 0.93 (d, *J* = 6.4 Hz, 3 H, 21-H_3), 0.93 (s, 3 H, 19-H_3), 2.63 (m, 1 H, 16α-H), 2.86 (dd, *J* = 10.3 and 3.0 Hz, 1 H, 26_{eq}-H), 3.10 (dd, *J* = 12.2 and 4.5 Hz, 1 H, 5α-H), 3.72 (m, w/2 = 12 Hz, 1 H, 2ß-H), 4.02 (m, w/2 = 5 Hz, 1 H, 3ß-H), 4.10 (m, 2 H, 7-H_2). – EIMS: *m/z* (%) = 445 (12) [M^+], 444 (10) [M^+– H], 430 (7) [$M^+ - CH_3$], 204 (38) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (12), 98 (12).

$C_{27}H_{43}NO_4$: Calcd. 445.3192, found 445.3180 (HRMS).

2α,3α-Dihydroxy-6,7-seco-5α,22αH,25ßH-solanidano-6,7-lactone N-Oxide (2α,3α-Dihydroxy-B-homo-7-oxa-5α,22αH,25ßH-solanidan-6-one N-Oxide, 16). Compound **16** was prepared in the same manner as described for **8** by treatment of 1.5 mg (0.0034 mmol) of **15** in 0.5 ml of dichloromethane with 3 mg (0.015 mmol) of 85 % MCPBA, but with a reaction time of 19 hs. 1.2 mg (77 %) of **16** as a colourless amorphous product with R_f = 0.83 (eluant c), which was directly used for the bioassay. – EIMS: *m/z* (%) = 461 (11.4) [M^+], 444 (12) [$M^+ - OH$], 430 (6) [$M^+ - O - CH_3$], 392 (1.5) [$M^+ - C_5H_9$], 204 (43) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 114 (7) [$C_6H_{12}NO^+$].

2α,3α-Dihydroxy-5,6-seco-5α,22αH,25βH-solanidano-6,5-lactone (2α,3α-Dihydroxy-B-homo-6-oxa-5α,22αH,25βH-solanidan-7-one, 18). The diacetate **17** (13 mg, 0.025 mmol) was hydrolysed in the same manner as the diacetate **14**: 8.0 mg (73 %) of **18** as colourless needles (from acetone) with mp 212-214 °C, $[\alpha]_D^{25}$ = + 42.2° (c = 0.030), and R_f = 0.25 (eluant b). – ^{1}H NMR (500 MHz): δ = 0.84 (d, *J* = 6.4 Hz, 3 H, 27-H_3), 0.85 (s, 3 H, 18-H_3), 0.93 (d, *J* = 6.4 Hz, 3 H, 21-H_3), 0.95 (s, 3 H, 19-H_3), 2.50 (m, 2 H, 7-H_2), 2.62 (m, w/2 = 12 Hz, 1 H, 16α-H), 2.83 (m, w/2 = 9 Hz, 1 H, 26_{eq}-H), 3.78 (m, w/2 = 13 Hz, 1 H, 2ß-H), 4.03 (m, w/2 = 5 Hz, 1 H, 3ß-H), 4.60 (dd, *J* = 11.3 and 5.2 Hz, 1 H, 5α-H). – EIMS: *m/z* (%) = 445 (15) [M^+], 444 (13) [M^+– H], 430 (8) [$M^+ - CH_3$], 204 (32) [$C_{14}H_{22}N^+$], 150 (100) [$C_{10}H_{16}N^+$], 136 (12), 98 (12).

Lactone 15 and its Isomer 18 by Direct Baeyer-Villiger Oxidation of the 2α,3α-Dihydroxy-6-ketone 10. Compound **10** (43 mg, 0.100 mmol) in 3 ml of dichloromethane was oxidized with trifluoroperoxyacetic acid in the presence of disodium hydrogen phosphate as described for the oxidation of compound **11**. After treatment with aqueous sodium hydrogen sulfite solution, the mixture was alkalized with potassium carbonate solution to pH = 9 and then extracted with dichloromethane (3 x 20 ml), washed with water and brine, dried with anhydrous sodium sulfate, and concentrated in vacuo. The residue [36 mg, TLC: 2 major spots with R_f = 0.30 and 0.25 (eluant b), **10** has R_f = 0.35] was separated by column chromatography on 12 g of silica gel and elution with chloroform/methanol (95:5) and then by preparative TLC (eluant b) yielding 23 mg (52 %) of compound **15** (R_f = 0.30) and 11 mg (25 %) of compound **18** (R_f = 0.25), which were shown to be identical in every respect with the above-described 2α,3α-dihydroxylactones **15** and **18**.

Bioassay

Rice Lamina Inclination Bioassay. This bioassay to determine the brassinosteroidal activity[14] was carried out according to the procedure published in ref.[15] (cf. also ref.[16]) using germinated seedlings of rice (*Oryza sativa* L. cv Koshihikari). The synthesized nitrogenous brassinosteroid analogues **10**, **12**, **15**, and **16** as well as 24-epibrassinolide [(24*R*)-**1**] as standard were tested by application of 0.1 ppm of the compounds in question against water as control. The possible antibrassinosteroidal activities were investigated by testing 1:1 mixtures of compounds **10** or **12** with 24-epicastasterone [(24*R*)-**2**] and **15** or **16** with 24-epibrassinolide [(24*R*)-**1**], respectively, using also 0.1 ppm concentrations. The calculated activities are means of the measured values of each ten repetitions.

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