New Route for the Synthesis of (22S,23S)-28-Homobrassinolide

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Abstract: A new and highly efficient synthesis of (22S,23S)-28-homobrassinolide (3) has been achieved. (2x,3x,22S,23S)-Tetrahydroxy-5x-stigmastan-6-one (4), a key intermediate for the synthesis of 3, and (2x,3x)-dihydroxy-5x-stigmast-22-en-6-one (6), a versatile intermediate for the synthesis of 3, and several naturally occurring brassinosteroids have been obtained in excellent yield from stigmaster-2,(22E)-dien-6-one (5) by catalytic RuO4 hydroxylation. Sodium perborate, a cheap and large-scale industrial chemical is used for Baeyer–Villiger oxidation of B-ring ketone 9 to its lactone 10.

Key words: brassinosteroids, dihydroxylation, plant growth promoters, ruthenium tetroxide, sodium perborate

Brassinolide 1 (I) and its analogues, collectively known as brassinosteroids,2 are a new class of steroidal phytohormones3 with high growth-promoting and anti-stress activity. Among the various naturally occurring brassinosteroids, brassinolide (I) is the most active,5,5 for inducing plant growth promoting activity in various bioassay systems. 28-Homobrassinolide (2), which differs from I only in the 24-alkyl group, is almost as active6 as I (Figure 1). The unnatural (22S,23S)-28-homobrassinolide (3), readily obtained7,8 from stigmasterol is moderately active. The minimal effect of 3 and 4 at 1 µg/plant and indoleacetic acid (IAA) at 0.1 µg/plant can be enhanced significantly by the co-application of both phytohormones.2) These findings are encouraging for further application of compound 3 in agriculture because it would allow for the enhancement of crop yields by the co-application of IAA and brassinosteroid 3, which is easier to synthesise at lower economic cost. Use of brassinolide (1) in the field trials and under practical cultivation conditions is found to be ineffective and disappointing.9 Under field conditions, the growing stages of plants are not uniform, and moreover, the flowering period of a community generally lasts from one to a few weeks. Accordingly, the prolonged activity of the compound is a very important factor. So, the instability of brassinolide (1) and its rapid metabolism in plants might be the reasons why the field trials were not effective as compared with those test results obtained in bioassay and greenhouse conditions. On the other hand the unnatural (22S,23S)-28-homobrassinolide (3) has a surprisingly high response in several plants in the field with respect to results10,11 obtained in bioassays. Brassinol steroid 3 has been commercialised and finds its application in enhancing the yields12 of a variety of agricultural crops. In continuation of our efforts10,11 towards the synthesis of brassinosteroids and due to the practical use of compound 3 for increasing food production we wish to report here a new and highly efficient route for the synthesis of (22S,23S)-28-homobrassinolide (3).

Figure 1 Structures of Brassinosteroids 1–3 and stigmastanone 4

Use of ruthenium tetroxide as an oxidant for organic functional group transformations14,15 has been well recognised. Ruthenium tetroxide is a very powerful oxidising agent that can be generated in situ from RuCl3·3H2O and an aqueous solution of NaIO4. While normally used to cleave oxidatively alkenes, ruthenium tetroxide has been shown to behave as a dihydroxyating agent16,17 under stringent reaction conditions. Ruthenium tetroxide has several practical advantages over osmium tetroxide as it is less volatile, less toxic, less expensive and readily available. It was questionable, however, whether reaction of 5 with ruthenium tetroxide would provide better selectivity than OsO4 or with a catalytic amount of OsO4 and N-methylmorpholine-N-oxide7,19 and if over-oxidation could be avoided to furnish a sufficient yield of the desired tetraol 4. To our delight, addition of an aqueous solution of catalytic amounts of RuO4 [the total solution is prepared from 0.14 mol equivalents of RuCl3·3H2O and an excess of NaIO4 (1.5 mol equiv) in water and divided into two equal lots] in 2 lots in quick succession to a solution of 2,22-dien-6-one 5 in ethyl acetate, acetone, acetonitrile at 4–6 °C for 6 minutes (see details in experimental section)
and quenching the reaction with sodium metabisulfite afforded the tetrahydroxy ketone 4 as a single isomer in 83% yield along with dihydroxy keto olefin 6 in 12% yield (Scheme 1). Hydroxylation of 5 with RuO₄ is highly susceptible to temperature, time and the solvent system used. Use of a two-phase solvent system of ethyl acetate, acetonitrile and water led to incomplete reaction and poor yield. In the solvent system acetonitrile is essential for avoiding the inactivation of the catalyst and to increase the effectiveness and reliability of the hydroxylation. Under low temperature (below 5 °C) the starting olefin 5 precipitated from the solvent system used in this hydroxylation. Although the hydroxylation of the 2,3-double bond in ring A of compound 5 with catalytic amount of OSO₄ and excess of N-methylmorpholine-N-oxide is completed in 5–7 h, the sterically hindered 22,23-double bond [due to 24 (S)-ethyl group and steroid ring D], after 4 days afforded the tetrahydroxy compound 4 in moderate yield. Thus in this case, ruthenium tetroxide oxidation of 5 to 4 is significantly superior to osmium tetroxide oxidation and it appears that this oxidation is kinetically controlled.

Addition of an aqueous solution of catalytic amounts of RuO₂ and 1.5 equivalents of NaO₂ to a solution of compound 5 in ethyl acetate, acetonitrile at 5 °C for 2 minutes furnished the 2α,3α-dihydroxy compound 6 in 92% yield. No trace of the tetrrol 4 was detected (300 MHz 1H NMR). This diol 6 is an important and versatile intermediate for the synthesis of a number of naturally occurring brassinosteroids including brassinolide (1), 28-homobrassinolide (2), 4,19-dolicholide and dolichosterone. 22 Diol 6 was synthesised earlier in low yield. The known diaclactonate 7 on treatment with catalytic amount of RuO₄ in 2 equal lots in quick succession at 6 °C for 5 minutes furnished the 2,3-diacetoxy-22,23-dihydroxy ketone 8 in 86% yield. Compound 8 on acetylation gave the tetraacetoxy ketone 9 in 94% yield.

RuO₄ reacts with olefins, like MnO₄⁻ and OsO₄, by stereospecific cis addition to produce initially a cyclic ruthenium(VI) ester which is unstable compared to the osmium(VI) ester. The stereochemistry of the tetrahydroxy groups in compound 4 was assigned on the basis of 11C NMR spectrum, which exhibited four signals due to C=O (δ = 67.97, 68.04, 70.25 and 71.94). The close similarity of the chemical shifts of compound 4 with the relevant shifts reported for analogous compounds confirm it as (2α,3α,22S,23S)-2,3,22,23-tetrahydroxy-5α-stigmastan-6-one (4).

Baeyer–Villiger oxidation of tetraacetate ketone 9 with excess of sodium perborate in trifluoroacetic acid at 60 °C for 6 hours afforded a crude product. Column chromatographic purification furnished the known 4,5,7,8 earlier. Saponification of 10 with K₂CO₃ in aqueous methanol, followed by acidification with aqueous 6 N HCl gave the expected (22S,23S)-28-homobrassinolide (3) in 84% yield. This compound 3 is found to be identical in all respects with the (22S,23S)-28-homobrassinolide (3) reported earlier.

In conclusion, we have achieved a highly efficient chemoselective and stereoselective method for dihydroxylation and tetrahydroxylation of the diene 5 with catalytic amount of RuO₄. Baeyer–Villiger oxidation of the tetraacetate 9 to lactone 10 with very cheap sodium perborate in moderate yield is also worth mentioning. This paves a way for large-scale preparation of this plant hormone, so that it may be used to increase the yield of a variety of agricultural crops.

All mps are uncorrected and measured on Yanaco Micro melting point apparatus. IR spectra were recorded as Nujol mull on a Shimadzu FTIR-8400 spectrophotometer. 1H and 13C NMR were recorded on a Varian Gemini 300 spectrophotometer. 1H and 13C NMR were recorded on a Varian Gemini 300 spectrophotometer.
corded on Bruker AC 200 (200 MHz) or msl 300 (300 MHz) spectrometer using CDCl₃ as solvent and TMS as an internal standard. J values are given in Hz. Mass spectra were recorded on Finnigan Mat 1020C spectrometer (70 eV). Optical rotations were measured on JASCO-181 Digital polarimeter using a sodium light (λ = 5893 Å) source. Elemental analyses were carried out in the Analytical Section of the Department. All solvents and reagents used were of commercial grade. Reactions were monitored by TLC using TLC aluminum sheets, silica gel 60F 254 precoated, Merck, Germany and locating the spots spraying with ethanolic solution of phosphomolybdic acid followed by heating. Usual workup means the organic extract was thoroughly washed with water and brine and finally dried over anhyd Na₂SO₄. Yields refer to crystallised material or homogenous products (TLC) obtained by column chromatography.

(2a,3a)-Diacyloxy-5α-stigmasteran-6-one (4) and Compound 6

A solution of RuO₄ was prepared by adding a solution of NaIO₄ (639 mg, 3.0 mmol) in H₂O (1.2 mL) all at once at 6–7 °C. The reaction mixture was stirred at 16 h. The mixture was poured into ice cold sat. NaHCO₃ solution and was stirred for 1 h. It was then extracted with EtOAc (3 × 50 mL). The organic layer was washed with H₂O (2 × 25 mL), ice cold 2N HCl (2 × 25 mL), H₂O (2 × 25 mL), and finally with brine (2 × 25 mL). EtOAc extract was dried (Na₂SO₄) and evaporated. Solvent was removed by evaporation, and the residue obtained by removing the solvent was purified by column chromatography on silica gel with hexane–EtOAc (9.8:0.2) to afford a pure crude diol 7. (2a,3a)-Diacetoxy-5α-stigmasteran-6-one was obtained as a colourless solid (53.1 mg, 12%). mp 234–235 °C (MeOH–CHCl₃).

6

IR (Nujol): 3473 (OH), 1743 (OCOCH₃), 1712 cm⁻¹ (C=O).

MS (70 eV): m/z (%) 428 [M⁺ – (CH₃CO₂H)₃], 388 [9, (447 – (OCOCH₃))], 346 [7, (529 – (CH₃CO₂H)₃)], 327 (9), 321 (7), 269 (5), 173 (9), 145 (17), 99 (38), 85 (50), 55 (100).

Anal. calc. for C₃₃H₅₄O₇: C, 70.18; H, 9.67.

All the solvents were evaporated and the residue was extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with H₂O (2 × 50 mL) and dried (Na₂SO₄). IR and locating the spots spraying with ethanolic solution of phosphomolybdic acid followed by heating. Usual workup means the organic extract was thoroughly washed with water and brine and finally dried over anhyd Na₂SO₄. Yields refer to crystallised material or homogenous products (TLC) obtained by column chromatography.
(2R,3R,22S,23S)-Tetraacetoxy-5α-stigmasteran-6-one (9) from 4 and 8

Compound 4 (478 mg, 1 mmol) in pyridine (2.5 mL) on treatment with Ac₂O (1.5 mL, 14.7 mmol) and N,N-dimethylaminopyridine (13.0 mg, 0.10 mmol) at 28 °C for 16 h afforded the known \(^9\) as a foam (620 mg, 96%). Following the same procedure, compound 8 (562 mg, 1.0 mmol) in pyridine (2.5 mL), Ac₂O (1.5 mL, 14.7 mmol), and N,N-dimethylaminopyridine (13.0 mg, 0.10 mmol) afforded 9 (594 mg, 92%).

IR (Nujol): 1742 (OCOCH₃), 1710 cm⁻¹ (C=O).

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(12) Godrej Agrovet Ltd. Mumbai, India, has commercialised (2S,23S)-homobrassinolide for the enhancement of yields of paddy, wheat, tomato, cabbage, cauliflower, potato, groundnut (peanut), cotton, grapes and tea.


