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TETRAHEDRON

Synthesis of two Analogues of Brassinolide, Possible Plant Growth Promoting Steroids

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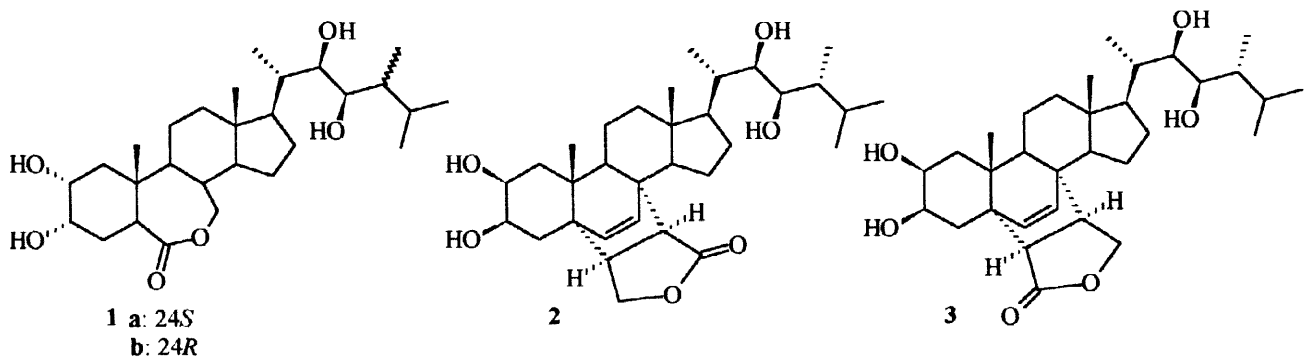
Abstract: The synthesis of two isomeric brassinosteroids, analogues of brassinolide, containing the 2β,3β diol system typical of ecdysteroids and a five-membered lactone bonded to the steroid ring B is described. The synthesis leads to both lactones *via* a metal hydride reduction of the Diels-Alder adduct of ergosterol.

INTRODUCTION

Brassinolide **1a** and its congeners are a group of steroids (over 40) known to elicit marked growth responses in plants.[1a-c] Brassinosteroids are similar in structure to ecdysteroids, the moulting hormones of insects and other arthropods,[1c] but (*inter alia*) the stereochemistry of the 2,3-diol system is the opposite.

The synthesis of brassinolide and some natural and unnatural congeners[2,3a-c] (like the 24*R* brassinosterol **1b**) has led to their ready availability and to a vast knowledge of the biological relevance of these steroids as stress modulators for plants and as ecdysteroid antagonists for insects.[1c] More recently, the coupling of molecular genetics and studies on the pathway of brassinolide biosynthesis[1a,4a-c] has established the hormonal status of brassinosterols creating renewed interest in the synthesis and the biological evaluation[5a-c] of brassinolide and its analogues. Thus we [6] decided to synthesize the two isomeric tetrahydroxylated lactones **2** and **3**.



1 **a**: 24*S*
b: 24*R*

2

3

These compounds possess a five-membered lactone group bonded to the steroidal ring B, the glycolic group typical of brassinosterol **1b** in the side chain and at positions 2 and 3 the diol with the β-configuration

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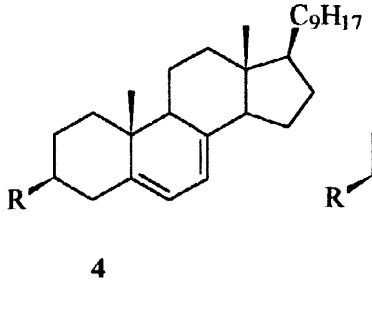
typical of ecdysteroids.[1c] The lactone group could might produce the biological activity of the natural compound while the β,β-stereochemistry of the 2,3-diol system could influence the antiecdysteroid activity.

RESULTS AND DISCUSSION

A short synthesis of the lactones **2** and **3** started with ergosterol **4a**, a readily available and inexpensive sterol. Ergosterol, *via* its adduct with maleic anhydride **5a** (Inhoffen adduct),[7-9] could lead to the steroid lactones **6a** and **7a**, possible precursors of the trienes **8** and **9** which are key intermediates to obtaining **2** and **3** by the Sharpless asymmetric dihydroxylation reaction (AD).[10,11] Since in previous work we had obtained the lactone **6a** by the metal hydride reduction of the Inhoffen adducts **5a** (or **5b**),[12] we prepared the triene **8** by dehydrotosylation of the 3β-*p*-toluenesulfonate **6e**, by treatment with *sym*-collidine at reflux.



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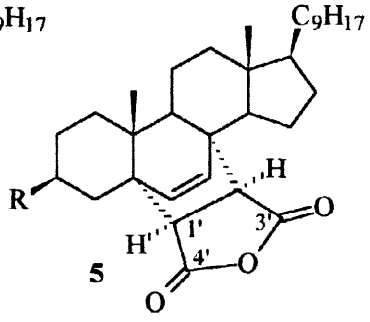

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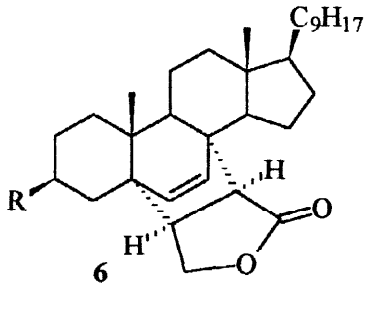

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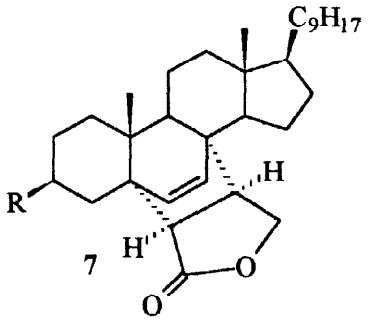
a: R = OH **d**: R = OSi(tBu)(Me)$_2$
b: R = OAc
c: R = OCH_3 **e**: R = OTs



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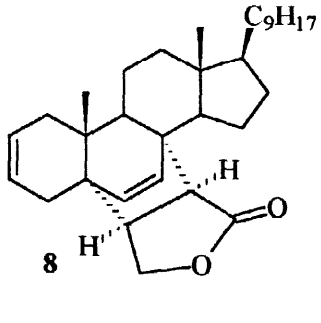
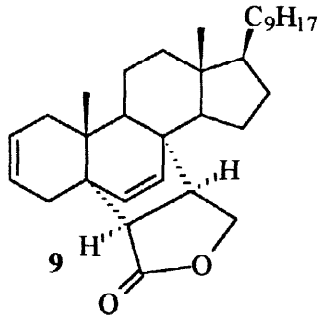


8



9

These conditions left the lactone group unaltered and afforded the Δ^2-lactone **8** in satisfactory yields (68%). Since compound **8** was accompanied by minor amounts of the undesired Δ^3-isomer, attempts were also made to eliminate the formation of this by-product. However using different basic conditions (K_2CO_3-CH_3CN; Py or Et_3N-CH_2Cl_2) greater amounts of this isomer were obtained.

The isomeric lactone **7**, precursor of triene **9**, was not obtained by reduction of the anhydrides **5a** or **5b**.[12] However, considering the possible mechanism of the reduction of **5a** and **5b**, and the factors influencing the course of the metal hydride reduction of cyclic anhydrides,[13-16] we thought that changing the 3β-hydroxy and 3β-acetoxy groups of **5a** and **5b** could modify the regioselectivity of these reductions. Strong support for these considerations was also derived from the results of Le Quesne *et al.*[13] who showed that the sodium borohydride reduction of the 3β-methyl ether adduct **5c**, possessing a less good directing effect on the hydride, affords lactones **6c** and **7c** in roughly equal amounts.

Considering that the 3β-dimethylthexylsilyloxy group was a substituent unable to direct hydride action and was suitable for the later elaboration that affords the triene **9**, we prepared the Diels-Alder adduct **5d** from 3β-dimethylthexylsilylergosterol **4d** and subjected it to reduction by various hydrides.

In effect its reduction with sodium borohydride or lithium aluminum hydride afforded the isomeric lactones **6d** and **7d** in a 1:1 ratio, thus confirming the important role of the nature of the 3β-substituent in the regioselectivity of the reduction. On the other hand, L and K Selectrides®, formed by a bulky hydride with two different counterions, led to the undesired lactone **6d** in a 6:4 ratio with the isomer **7d**, regardless of both the temperature (- 50 °C or 23 °C) and the hydride counterion (Li^+ or K^+). Thus we decided to pursue the synthesis of the desired trienic lactone **9** *via* the lactone **7d** obtained by sodium borohydride reduction of **5d**. The silyl derivative **7d** was treated with hydrofluoric acid to afford the hydroxylactone **7a** which was then transformed into trienelactone **9** by a reaction sequence (tosylation and dehydrotosylation) similar to that used to obtain its isomer **8** (53% yield).

The two triene lactones **8** and **9** are easily identified by their ^1H NMR spectra: compound **8** shows a signal at 2.52 ppm characteristic of the 15α-H which is deshielded by the lactone carbonyl; the spectrum of the lactone **9** shows no such signal. Instead signals can be seen at 3.22 ppm for the deshielded 4α-H and 5.73 ppm for the deshielded 3-vinylic proton, the two protons being coupled (in compound **8** the same 3-vinylic proton resonates at 5.68 ppm). The doublet due to the proton adjacent to the lactone carbonyl, resonating at 2.41 ppm in the lactone **8** spectrum and at 2.77 ppm in the lactone **9**, discriminates between the two lactones.

During the synthesis, the trienes **8** and **9** were treated separately with AD-mix-β in an asymmetric dihydroxylation of Sharpless which, in principle and on the basis of results previously reported by T. C. McMorris,[11] could introduce in the steroid side chain a diol system with the appropriate 22*R*,23*R* stereochemistry and a diol system at the 2β,3β position. In fact, we were confident that dihydroxylation of the Δ^2 double bond could take place on the β-side of the molecule due to the steric hindrance by the pendant lactone group bonded to the 5α,8α-positions; this hindrance should exceed that of the C-19 methyl group located in the β side of the molecule. In fact, separate reactions of the triene lactones **8** and **9** with AD-mix-β proceeded slowly (7 days), affording the desired tetraols of the expected structures **2** and **3**. Structure was assigned on the basis of

the known reactivity of AD-mix-β and ^{1}H NMR spectra analysis. The 2β,3β-configuration for the 2,3-diol system of **3** was evident from the observed values of the coupling constants of ring A protons. In fact the axial 4β-H shows two large coupling constant values: one for the geminal coupling with the 4α-H (*J* 13.0 Hz; deshielded by the lactone group) and the other (*J* 12.0 Hz, calcd[17] 11.5 Hz) due to the *trans*-diaxial coupling with the 3α-H. Moreover, the equatorial 2α-H shows three small coupling constants, due to coupling with the 1α and 3α axial protons (*J* 5.0 Hz, calcd[17] 5.1 Hz; *J* 3.5 Hz, calcd[17] 4.2 Hz) and the equatorial 1β-H (*J* 2.5 Hz, calcd[17] 3.2 Hz).

Assuming a chair conformation for the ring A, this pattern of coupling constants is consistent only with the structure **3**. This conformation is suggested by the simple consideration that ring B is forced into a boat conformation and by MM2 calculations that show a minor energy content (10 kcal mol^{-1}) for this conformation with respect to a hypothetical twisted ring A. Similar simple considerations allow the assigning of the tetraol lactone **2** structure.

EXPERIMENTAL

^{1}H NMR spectra (500.13 MHz) were recorded in $CDCl_3$ at 303 K and were referenced to $CHCl_3$ at 7.24 ppm, *J* values are given in Hz. Mass spectra (electron impact) were determined on a Hewlett Packard 5988A spectrometer by direct inlet. Optical rotations were measured for 1% $CHCl_3$ solutions, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Column chromatography refers to flash chromatography.[18] Hexane-ethyl acetate mixtures were used as TLC developing solvents. Usual work-up refers to washing the organic layer with water, drying it over anhydrous Na_2SO_4, and evaporating the solvent under reduced pressure.

3β-Tosyloxylactone **6e**

The hydroxylactone **6a**[12] (444 mg, 0.92 mmol) dissolved in pyridine (8 cm^3) was treated with *p*-toluenesulfonyl chloride (514 mg, 2.70 mmol) at 25 °C for 24 h. The reaction mixture was poured into ice-cold aqueous HCl (1:4 v/v) and extracted with ethyl acetate. After usual work-up the crude desired compound **6e** was obtained, as a white solid, (425 mg, 72%): mp 180-182 °C (from diisopropyl ether); $[\alpha]_D^{20}$ - 36.8; ν_{max} 1740, 1370 cm^{-1}; ^{1}H NMR δ 7.77 (2 H, d, *J* 8.0, aromatics), 7.34 (2 H, d, *J* 8.0, aromatics), 6.20 (1 H, d, *J* 9.1, 7-H), 5.68 (1 H, d, *J* 9.1, 6-H), 5.22-5.13 (2 H, m, 22-H and 23-H), 4.56 (1 H, dddd, *J* 11.0, 11.0, 6.0 and 6.0, 3α-H), 3.88 (1 H, dd, *J* 9.0 and 9.0, 4'β-H), 3.55 (1 H, dd, *J* 9.0 and 5.5, 4'α-H), 2.96 (1 H, ddd, *J* 10.0, 9.0 and 5.5, 1'-H), 2.53 (1 H, dddd, *J* 12.5, 9.8, 7.9 and 3.5, 15α-H), 2.44 (3 H, s, CH_3Ph), 2.38 (1 H, d, *J* 10.0, 2'-H), 0.85 (3 H, s, 19-Me), 0.68 (3 H, s, 18-Me). (Found: C, 74.1; H, 8.7. Calcd for $C_{39}H_{54}O_5S$: C, 73.78; H, 8.57%).

Trienelactone **8**

The lactone **6e** (600 mg, 0.94 mmol) dissolved in *sym*-collidine (2 cm^3) was refluxed for 4 h. The reaction mixture was poured into ice-cold aqueous HCl (1:4 v/v) and extracted with ethyl acetate. Usual work-up and purification by argentic column chromatography [silica gel G/Celite/$AgNO_3$ (1:1:0.2) eluting with hexane-ethyl acetate; 100:3, v/v] afforded the desired compound **8**, as a white solid, (297 mg, 68%): mp 132-133 °C; $[\alpha]_D^{20}$ - 132.4; ν_{max} (KBr) 1760 cm^{-1}; ^1H NMR δ 6.26 (1 H, d, *J* 9.1, 7-H), 5.82 (1 H, d, *J* 9.1, 6-H), 5.68 (1 H, m, 3-H), 5.57 (1 H, m, 2-H), 5.22-5.14 (2 H, overlapping, 22-H and 23-H), 4.05 (1 H, dd, *J* 9.2 and 9.2, 4'β-H), 3.78 (1 H, dd, *J* 9.2 and 6.0, 4'α-H), 3.04 (1 H, ddd, *J* 9.7, 9.2 and 6.0, 1'-H), 2.53 (1 H, dddd, *J* 12.5, 9.8, 7.9 and 3.5, 15α-H), 2.42 (1 H, d, *J* 9.7, 2'-H), 0.78 (3 H, s, 19-Me), 0.76 (3 H, s, 18-Me); *m/z* 462 (M^+, 10%), 447 (5), 378 (100), 253 (56). (Found: C, 83.2; H, 9.9. Calcd for $C_{32}H_{46}O_2$: C, 83.06; H, 10.02%). In the first fractions the undesired Δ^3 isomer (109 mg, 25%) was obtained: mp 128-130 °C; ν_{max} (KBr) 1763 cm^{-1} ^1H NMR δ 6.29 (1 H, d, *J* 9.0, 7-H), 5.94 (1 H, d, *J* 9.0, 6-H), 5.81-5.73 (2 H, overlapping, 3-H and 4-H), 5.22-5.15 (2 H, overlapping, 22-H and 23-H), 4.09 (1 H, dd, *J* 9.0, 9.0 and 4'β-H), 3.90 (1 H, dd, *J* 9.0 and 5.0, 4'α-H), 2.92 (1 H, ddd, *J* 10.1, 9.0 and 5.0, 1'-H), 2.60 (1 H, dddd, *J* 12.5, 9.8, 8.0 and 3.5, 15α-H), 2.44 (1 H, d, *J* 10.1, 2'-H), 0.78 (3 H, s, 19-Me), 0.72 (3 H, s, 18-Me); *m/z* 462 (M^+, 1%), 447 (0.3), 378 (100), 253 (19).

3β-Dimethylthexylsilyloxyergosta-5,7,22-triene **4d**

To a solution of ergosterol **4a** (5.0 g, 12.6 mmol) in dimethylformamide (150 cm^3), imidazole (2.28 g, 33.5 mmol) and dimethylthexylchlorosilane (5.0 cm^3; 25.4 mmol) were added under stirring and the solution was kept overnight at 25 °C. At this time the solution was diluted with ice cold water and the solid was filtered and washed with water. After crystallization from ethanol the desired compound **4d** (5.53 g, 82%) was obtained, as a white solid: mp 134-135 °C; $[\alpha]_D^{20}$ - 45.1; ^1H NMR δ 5.52 (1 H, dd, *J* 5.0 and < 1.0, 7-H), 5.36 (1 H, ddd, *J* 5.0, 1.0 and 1.0, 6-H), 5.23-5.13 (2 H, overlapping, 22-H and 23-H), 3.56 (1 H, dddd, *J* 11.2, 11.2, 6.0 and 6.0, 3α-H), 0.92 (3 H, s, 19-Me), 0.61 (3 H, s, 18-Me). (Found: C, 80.1; H, 11.4. Calcd for $C_{36}H_{62}OSi$: C, 80.23; H, 11.59%).

3β-Dimethylthexylsilyloxy-5α,8α-ethanoergosta-6,22-diene-1'β,2'β-dicarboxylic acid anhydride **5d**

3β-Dimethylthexylsilyloxyergosta-5,7,22-triene **4d** (5.0 g, 9.27 mmol), dissolved in xylene (50 cm^3), was treated with maleic anhydride (2.7 g, 27.6 mmol) under argon at 135 °C for 2 h. After usual work-up, the crude residue was chromatographed (hexane-ethyl acetate; 100:5, v/v) to afford the anhydride **5d**, as a white solid, (3.95 g, 67%) mp 252-253 °C (from diisopropyl ether); $[\alpha]_D^{20}$ - 25; ν_{max} (KBr) 1850, 1775, 1370 cm^{-1}; ^1H NMR δ 6.21 (1 H, d, *J* 9.1, 7-H), 5.78 (1 H, d, *J* 9.1, 6-H), 5.23-5.14 (2 H, overlapping, 22-H and 23-H), 4.07 (1 H, dddd, *J* 11.0, 11.0, 6.0 and 6.0, 3α-H), 3.42 (1 H, d, *J* 9.0, 1'-H), 2.82 (1 H, d, *J* 9.0, 2'-H), 2.53 (1 H, ddd, *J*

13.1, 6.0 and 2.1, 4α-H), 2.44 (1 H, dddd, *J* 12.5, 10.0, 7.0 and 3.5, 15α-H), 0.92 (3 H, s, 19-Me), 0.71 (3 H, s, 18-Me); *m/z* 551 (35%), 453 (32), 377 (100). (Found: C, 75.35; H, 10.2. Calcd for $C_{40}H_{64}O_4Si$: C, 75.42; H, 10.13%).

Reduction of 3β-dimethylthexylsilyloxy-5α,8α-ethanoergosta-6,22-diene-1'β,2'β-dicarboxylic acid anhydride **5d**

(a) Reduction with sodium borohydride. The anhydride **5d** (300 mg, 0.47 mmol) dissolved in anhydrous THF (15 cm^3) was treated with $NaBH_4$ (17.8 mg, 0.47 mmol), under stirring at room temperature for 12 h. The mixture was acidified with HCl (2 M) and extracted with ethyl acetate to afford, after usual work-up and rapid chromatography (hexane-ethyl acetate; 100:5, v/v), first the dimethylthexylsilyloxylactone **6d**, as a white solid, (134 mg, 46%): mp 252-253 °C; $[\alpha]_D^{20}$ - 85.3; ν_{max} (KBr) 1760 cm^{-1}; ^1H NMR δ 6.21 (1 H, d, *J* 9.1, 7-H), 5.75 (1 H, d, *J* 9.1, 6-H), 5.23-5.14 (2 H, overlapping, 22-H and 23-H), 4.12 (1 H, dd, *J* 9.1 and 9.1, 4'β-H), 3.84 (1 H, dddd, *J* 11.0, 11.0, 6.0 and 6.0, 3α-H), 3.82 (1 H, dd, *J* 9.1 and 6.5, 4'α-H), 3.14 (1 H, ddd, *J* 10.5, 9.1 and 6.5, 1'-H), 2.55 (1 H, dddd, *J* 12.5, 10.0, 7.0 and 3.5, 15α-H), 2.40 (1 H, d, *J* 10.5, 2'-H), 0.89 (3 H, s, 19-Me), 0.71 (3 H, s, 18-Me). (Found: C, 77.0; H, 10.6. Calcd for $C_{40}H_{66}O_3Si$: C, 77.11; H, 10.68%).

Further elution afforded the dimethylthexylsilyloxylactone **7d**, as a white solid, (138 mg, 47%): mp 261-262 °C; $[\alpha]_D^{20}$ + 4.1; (KBr) 1760 cm^{-1} ^1H NMR δ 6.10 (1 H, d, *J* 9.1, 7-H), 5.83 (1 H, d, *J* 9.1, 6-H), 5.22-5.11 (2 H, overlapping, 22-H and 23-H), 4.12-4.05 (2 H, overlapping, 3α-H and 3'β-H), 3.70 (1 H, dd, *J* 9.1 and 6.0, 3'α-H), 2.96 (1 H, d, *J* 10.5, 1'-H), 2.56 (1 H, ddd, *J* 12.5, 6.0 and 2.2, 4α-H), 2.45 (1 H, ddd, *J* 10.5, 9.1 and 6.0, 2'-H), 0.90 (3 H, s, 19-Me), 0.74 (3 H, s, 18-Me). (Found: C, 77.2; H, 11.8. Calcd for $C_{40}H_{66}O_3Si$: C, 77.11; H, 10.68%).

(b) Reduction with lithium aluminum hydride. The anhydride **5d** (500 mg: 0.78 mmol) was dissolved in anhydrous THF (20 cm^3) and treated with $LiAlH_4$ (90 mg, 2.37 mmol) under stirring at 0 °C for 2 h. At this time ethyl acetate was added followed by water and the mixture was acidified with HCl (2 M). Usual work-up and chromatographic purification afforded the lactones **6d** (186 mg, 38%) and **7d** (176 mg, 36%), as white solids identical in all respects to those described above.

(c) Reduction with L and K Selectride.® The anhydride **5d** (300 mg, 0.47 mmol) was dissolved in dry, freshly distilled THF (15 cm^3). The solution was cooled to - 50 °C under argon. Selectride® (1.4 cm^3 of a 1 M solution in THF, 1.4 mmol) was injected slowly into the flask. The reaction was stirred for 2 h, then the temperature was allowed to rise slowly (3 h) to 23 °C. At this time NaOH (0.7 cm^3 of an aqueous 4 M solution) and H_2O_2 (1 cm^3 of a 30% solution) were added and stirring was continued overnight. The reaction was then acidified with HCl (2 M) and extracted with ethyl acetate. ^1H NMR inspection of the crude mixture, obtained after usual work-up, showed the presence of both lactones **6d** and **7d** in a 6:4 ratio. After chromatography the compounds were obtained in total 80% yield in the same ratio.

The yields and the ratio of compounds **6d** and **7d** were comparable with L and K Selectride® also at 23 °C.

3β-Hydroxylactone **7a**

The silyloxylactone **7d** (500 mg, 0.80 mmol), dissolved in THF (10 cm^3), was treated with aqueous HF (0.5 cm^3 of a 48% solution) at room temperature for 1 h. The solution was concentrated under reduced pressure, diluted with water and extracted with ethyl acetate. After usual work-up the crude desired compound **7a** was obtained, as a white solid, (300 mg, 78%): mp 224-226 °C (from diisopropyl ether); $[\alpha]_D^{23}$ +8.3; with all physicochemical properties identical with those reported.[12]

3β-Tosyloxylactone **7e**

The hydroxylactone **7a** (533 mg, 1.11 mmol) dissolved in pyridine (9 cm^3) was treated with *p*-toluenesulfonyl chloride (617 mg, 3.2 mmol) at 25 °C for 24 h. The reaction mixture was poured into ice-cold aqueous HCl (1:4 v/v) and extracted with ethyl acetate. After usual work-up the desired compound **7e** was obtained, as a white solid, (527 mg, 75%): mp 177-178 °C (from methanol); (KBr) 1760, 1370 cm^{-1}; ^1H NMR δ 7.86 (2 H, d, *J* 8.0, aromatics), 7.30 (2 H, d, *J* 8.0, aromatics), 6.11 (1 H, d, *J* 9.0, 7-H), 5.75 (1 H, d, *J* 9.0, 6-H), 5.19 (1 H, dd, *J* 15.0 and 7.0, 22-H or 23-H), 5.13 (1 H, dd, *J* 15.0 and 8.0, 22-H or 23-H), 4.95 (1 H, dddd, *J* 11.0, 11.0, 6.0 and 6.0, 3α-H), 4.08 (1 H, dd, *J* 9.0 and 9.0, 3'β-H), 3.66 (1 H, dd, *J* 9.1 and 5.5, 3'α-H), 2.82 (1 H, d, *J* 9.5, 1'-H), 2.58 (1 H, ddd, *J* 13.0, 6.0 and <1, 4α-H), 2.43 (1 H, ddd, *J* 9.5, 9.1 and 5.8, 2'-H), 2.41 (3 H, s, C*H*$_3$Ph), 0.88 (3 H, s, 19-Me), 0.72 (3 H, s, 18-Me). (Found: C, 73.6; H, 8.7. Calcd for $C_{39}H_{54}O_5S$: C, 73.78; H, 8.57%).

Trienelactone **9**

The lactone **7e** (800 mg, 1.26 mmol) dissolved in *sim*-collidine (3 cm^3) was refluxed for 4 h. The reaction mixture was then poured into ice-cold aqueous HCl (1:4, v/v) and extracted with ethyl acetate. Usual work-up and purification by argentic column chromatography [silica gel G/Celite/$AgNO_3$ (1:1:0.2, w/w/w) eluting with hexane-ethyl acetate; 100:3, v/v] afforded the desired compound **9**, as a white solid, (414 mg, 71%): mp 120-122 °C; $[\alpha]_D^{20}$ + 57.8; (KBr) 1760 cm^{-1}; ^1H NMR δ 6.19 (1 H, d, *J* 9.1, 7-H), 5.89 (1 H, d, *J* 9.1, 6-H), 5.72 (1 H, m, 3-H), 5.63 (1 H, m, 2-H), 5.21 (1 H, dd, *J* 15.0 and 7.5, 22-H or 23-H), 5.14 (1 H, dd, *J* 15.0 and 8.0, 22-H or 23-H), 4.12 (1 H, dd, *J* 9.1 and 9.1, 3'β-H), 3.76 (1 H, dd, *J* 9.1 and 5.8, 3'α-H), 3.21 (1 H, dd, *J* 18.0 and 6.1, 4α-H), 2.77 (1 H, d, *J* 9.5, 1'-H), 2.52 (1 H, ddd, *J* 9.5, 9.1 and 5.8, 2'-H), 0.79 (3 H, s, 19-Me), 0.77 (3 H, s, 18-Me). (Found: C, 83.2; H, 9.9. Calcd for $C_{32}H_{46}O_2$: C, 83.06; H, 10.02%). In the first fractions the undesired Δ^3 isomer (128 mg, 22%) was obtained.

Tetrahydroxylactones **2** *and* **3** *via asymmetric dihydroxylation (AD) reaction. General procedure*

AD Reactions were performed on 1 mmol scale of trienelactone **8** or **9** using the following experimental conditions reported by Sharpless.[10] A mixture of *tert*-butyl alcohol (10 cm^3), water (10 cm^3) and AD-mix-β (2.8 g) was stirred for few minutes at room temperature until two clear phases were produced. Methanesulphonamide (190 mg, 2 mmol) was then added and the mixture was cooled to -5 °C before adding the trienelactone **8** or **9** (1 mmol). The mixture was then stirred at 23 °C for two weeks. Sodium metabisulphite (3.0 g) was then added to the cold reaction mixture and the suspension was stirred for 30 min at room temperature. Extraction with trichloromethane, usual work-up and chromatographic purification (eluting with dicloromethane-methanol; 95:5, v/v) afforded the final tetraol **2** or **3**.

Trienelactone **8** (460 mg) afforded the tetraol **2** (411 mg, 78%), as a white solid, showing : mp 293-294 °C (triturated with methanol); (KBr) 3400, 1700, cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.12 (1 H, d, *J* 8.0, 7-H), 5.68 (1 H, d, *J* 8.0, 6-H), 4.35 (1 H, d, *J* 5.4, 3-O*H*), 4.31 (1 H, d *J* 2.0, 2-OH), 4.17 (1 H, d, *J* 6.0, 22-O*H*), 4.05 (1 H, dd, *J* 9.4 and 9.4, 4'β-H), 4.00 (1 H, d, *J* 6.0, 23-O*H*), 3.81 (1 H, dddd, *J* 3.5, 3.1, 3.1 and 2.0, 2α–H), 3.79 (1 H, dd, *J* 9.4 and 4.7, 4'α-H), 3.68 (1 H, dddd, *J* 11.9, 5.4, 5.0 and 3.5, 3α–H), 3.62 (1 H, ddd, *J* 10.0, 9.4 and 4.7, 1'-H), 3.49 (1 H, ddd, *J* 6.0, 5.4 and < 1, 22-H), 3.14 (1 H, ddd, *J* 6.0, 5.4 and 4.0, 23-H), 2.54 (2 H, overlapping, 15α-H and 2'-H), 1.94 (1 H, dd, *J* 15.5 and 3.1, 1β-H), 1.85 (1 H, dd, *J* 15.5 and 3.1, 1α-H),1.63 (1 H, dd, *J* 11.9 and 11.9, 4β-H), 1.52 (1 H, dd, *J* 11.9 and 5.0, 4α-H), 0.87 (3 H, d, *J* 6.9, 21-Me), 0.83 (3 H, d, *J* 6.9, 26- or 27-Me), 0.78 (3 H, d, *J* 6.9, 26 or 27-Me), 0.75 (3 H, s, 19-Me), 0.74 (3 H, d, *J* 6.9, 28-Me), 0.63 (3 H, s, 18-Me). (Found: C, 72.3; H, 9.7. Calcd for $C_{32}H_{50}O_6$: C, 72.42; H, 9.50%).

Trienelactone **9** (460 mg) afforded the tetraol **3**, as a white solid, (401 mg, 76%) showing: mp 248-250 °C(decomp.; triturated with methanol); (KBr) 3400, 1700, cm^{-1}; ^1H NMR δ 6.17 (1 H, d, *J* 8.5, 7-H), 5.87 (1 H, d, *J* 8.5, 6-H), 4.17 (1 H, ddd, *J* 12.0, 4.5 and 3.5, 3α–H), 4.14 (1 H, dd, *J* 9.5 and 9.5, 3'β-H), 4.06 (1 H, ddd, *J* 5.0, 3.5 and 2.5, 2α-H), 3.73 (1 H, dd, *J* 9.5 and 7.0, 3'α-H), 3.70 (1 H, dd, *J* 4.5 and < 1, 22-H), 3.48 (1 H, dd, *J* 6.5 and 4.5, 23-H), 2.90 (1 H, d, *J* 10.5, 1'-H), 2.48 (1 H, ddd, *J* 10.5, 9.5 and 7.0, 2'-H), 2.36 (1 H, dd, *J* 13.0 and 4.5, 4α-H), 2.13 (1 H, dd, *J* 13.0 and 12.0, 4β-H), 2.11 (1 H, dd, *J* 13.0, 2.5, 1β-H), 1.07 (3 H, s, 19-Me), 0.94 (3 H, d, *J* 6.5, 21-Me), 0.90 (3 H, d, *J* 7.0, 28-Me), 0.85 (3 H, d, *J* 7.0, 26- or 27-Me), 0.83 (3 H, d, *J* 7.0, 26- or 27-Me), 0.73 (3 H, s, 18-Me). (Found: C, 72.5; H, 9.4. Calcd for $C_{32}H_{50}O_6$: C, 72.42; H, 9.50%).

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