ELSEVIER

Chemistry and Physics of Lipids
103 (1999) 117–123

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

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Synthesis of 5α,8α-ethanoergosta-2,6,22-triene-2′β-hydroxymethylene-1′β-carboxylic acid lactone, a key intermediate for the synthesis of a brassinolide analogue

Pietro Allevi *, Alessandra Longo, Mario Anastasia

Dipartimento di Chimica e Biochimica Medica, Università degli Studi di Milano, Via Saldini 50, 20133 Milan, Italy

Received 3 February 1999; received in revised form 19 July 1999; accepted 19 July 1999

Abstract

Metal hydride reduction of the 5α,8α-ethanoergosta-2,6,22-triene-1′β,2′β-dicarboxylic acid anhydride prepared by a new route afforded in prevalence the corresponding 4′-lactone, a known steroidal intermediate affording a brassinolide congener by hydroxylation with osmium tetraoxide. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Brassinosteroids; Steroidal lactones; Inhoffen adducts; Hydride reduction

1. Introduction

In a recent paper from our laboratory (Allevi et al., 1999) we reported a simple synthesis of the two steroidic lactones **1** and **2** (Fig. 1) having some structural features of brassinosteroids, plant growth hormones, and of ecdysteroids, the moulting hormones of insects (Cutler et al., 1991; Yokota, 1997). Compounds **1** and **2** are prepared (Allevi et al., 1999) starting from the unsaturated lactones **3** and **4** which derive from the anhydrides **5a–c** (Inhoffen adducts of ergosterol) (Inhoffen, 1934; Birckelbaw et al., 1970). In particular, the lactone group of **3** is obtained, in a regioselective way, by reduction with metal hydrides of the Inhoffen adducts **5a** or **b**, probably since the 3β-substituents direct the reductions to the nearest carbonyl (Allevi et al., 1997; Rickard and Le Quesne, 1998). On the contrary, the lactone group of the compound **4** is obtainable only by sodium borohydride reduction of the 3β-thexyldimethylsilyloxy anhydride **5c** (Allevi et al., 1999) in a reaction in which an equal amount of the regioisomer lactone parent of **3** is also formed.

Here we report a more satisfactory synthesis of the lactone **4** and consequently the brassinosteroid **2**. This steroid is useful for continue some preliminary studies in which it has shown (unpublished results) some ability to stimulate the lamina inclination of rice (Maeda, 1965; Wada et al., 1981).

* Corresponding author. Tel.: +39-2-70645231; fax: +39-2-2361407.

E-mail address: pietro.allevi@unimi.it (P. Allevi)

PII: S0009-3084(99)00100-0

2. Materials and methods

2.1. General

All chemical materials were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). $NaBH_4$ was crystallised from 2-methoxyethyl ether and dried under vacuum.

Proton nuclear magnetic resonance spectra were recorded as $CDCl_3$ solution at 303 K on Bruker AM-500 spectrometer operating at 500.13 MHz. All chemical shifts are reported in ppm relative to $CHCl_3$ fixed at 7.24 ppm. Signal multiplicity was designated according to the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

All reactions and chromatographic separations were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60 F_{254}) using UV light or 50% sulphuric acid and heat as developing agent. E Merck 230-400 mesh silica gel was used for flash column chromatography (Still et al., 1978).

Usual work-up refers to washing the organic layer with water, drying over anhydrous Na_2SO_4 and evaporating the solvent under reduced pressure.

2.2. Dehydration of the hydroxyanhydride 5a

2.2.1. Tosylation of the hydroxyanhydride 5a

The hydroxyanhydride **5a** (Inhoffen, 1934; Birckelbaw et al., 1970) (0.9 g; 1.8 mmol), dissolved in pyridine, (10 ml) was treated with *p*-toluenesulfonyl chloride (1.03 g; 5.4 mmol) at 0°C. The mixture was then allowed to warm to 23°C and was stirred for a further 20 h. The mixture was diluted with an ice cold aqueous solution of HCl (5 M; 30 ml) and filtered. The crude solid obtained was dissolved in ethyl acetate (30 ml) and worked-up to afford the crude *p*-toluenesulfonyloxyanhydride **5d** (0.870 g; Y 74%): m.p. 181–182°C (from diisopropyl ether); TLC R_f 0.26 (eluting with hexane–ethyl acetate; 70:30, v/v); $[\alpha]_D^{20}$ +9.3 ($CHCl_3$, *c* 1); ^1H NMR δ 7.32 (2H, d, *J* 8.0 Hz, aromatics), 7.83 (2H, d, *J* 8.0 Hz, aromatics), 6.21 (1H, d, *J* 9.1 Hz, H-7), 5.70 (1H,

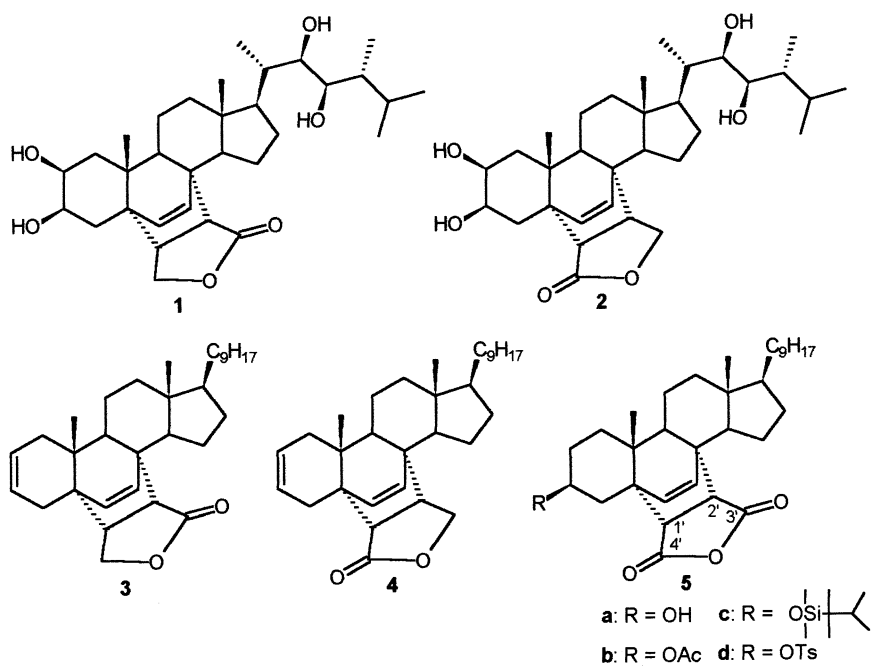

Fig. 1. Brassinolide analogues and their synthetic precursors.

d, *J* 9.1 Hz, H-6), 5.22-5.13 (2H, overlapping, H-22 and H-23), 4.88 (1H, dddd, *J* 10.5 Hz, 10.5, 6.1 and 5.0 Hz, H-3α), 3.26 (1H, d, *J* 8.5 Hz, H-2′), 2.82 (1H, d, *J* 8.5 Hz, H-1′), 2.53 (1H, ddd, *J* 13.0 Hz, 5.0 and 1.5 Hz; H-4α), 2.42 (3H, s, CH_3-Ar), 2.40 (1H, dddd, *J* 13.0, 10,0, 7.2 and 3.5 Hz, H-15α), 0.99 (3H, d, *J* 6.5 Hz, 21-CH_3), 0.90 (3H, s, 19-CH_3), 0.89 (3H, d, *J* 5.0 Hz, 28-CH_3), 0.81 (3H, d, *J* 7.0 Hz, 26-CH_3 or 27-CH_3), 0.79 (3H, d, *J* 7.0 Hz, 27-CH_3 or 26-CH_3), 0.69 (3H, s, 18-CH_3). Anal. Calcd. for $C_{39}H_{52}O_6S$: C 72.19, H 8.08. Found: C 73.7, H, 8.2.

2.2.2. Dehydrotosylation of p-toluenesulfonyloxyanhydride 5d

The *p*-toluenesulfonyloxyanhydride **5d** (800 mg; 1.2 mmol) dissolved in *sym*-collidine (1.5 ml) was refluxed for 8 h under argon. At this time the mixture was diluted with an ice cold aqueous solution of HCl (2 M; 10 ml), extracted with ethyl acetate (15 ml) and worked-up to afford, after purification by column chromatography (eluting with hexane–ethyl acetate; 90:10, v/v), the 5α,8α-ethanoergosta-2,6,22-triene-1′β,2′β-dicarboxylic acid anhydride **6** (340 mg; *Y* 59%): m.p. 118–119°C; $[\alpha]_D^{20}$ +0.10 ($CHCl_3$, *c* 1); ^{1}H NMR δ 6.22 (1H, d, *J* 9.0 Hz, H-7), 5.94 (1H, d, *J* 9.0 Hz, H-6), 5.74–5.66 (2H, overlapping, H-2 and H-3), 5.23–5.14 (2H, overlapping, H-22 and H-23), 3.27 (1H, d, *J* 8.5 Hz, H-2′), 3.25 (1H, ddd, *J* 13.0 Hz, 5.5 and 2.0 Hz; H-4α), 2.85 (1H, d, *J* 8.5 Hz, H-1′), 1.00 (3H, d, *J* 6.7 Hz, 21-CH_3), 0.90 (3H, d, *J* 7.0 Hz, 28-CH_3), 0.82 (3H, d, *J* 7.0 Hz, 26-CH_3 or 27-CH_3), 0.81 (3H, d, *J* 7.0 Hz, 27-CH_3 or 26-CH_3), 0.80 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3). Anal. Calcd. for $C_{32}H_{44}O_3$: C, 80.63; H, 9.30. Found: C, 80.5, H, 9.4.

Further elution yielded the 5α,8α-ethanoergosta-3,6,22-triene-1′β,2′β-dicarboxylic acid anhydride **7** (183 mg; *Y* 32%): m.p. 150–152°C (from diisopropyl ether); $[\alpha]_D^{20}$ −13.8 ($CHCl_3$, *c* 1); ^{1}H NMR δ 6.28 (1H, d, *J* 9.0 Hz, H-7), 6.07 (1H, ddd, *J* 10.5, 2.5 and 2.5 Hz, H-4), 6.00 (1H, d, *J* 9.0 Hz, H-6), 5.84 (1H, ddd, *J* 10.5, 3.5 and 3.5 Hz, H-3), 5.21–5.13 (2H, overlapping, H-22 and H-23), 3.29 (1H, d, *J* 8.5 Hz, H-2′), 2.84 (1H, d, *J* 8.5 Hz, H-1′), 2.43 (1H, dddd, *J* 13.0, 10.0, 7.2 and 3.5 Hz, H-15α), 1.01 (3H, d, *J* 6.5 Hz, 21-CH_3), 0.90 (3H, d, *J* 7.0 Hz, 28-CH_3), 0.83 (3H, s, 19-CH_3), 0.82 (3H, d, *J* 6.8 Hz, 26-CH_3 or 27-CH_3), 0.81 (3H, d, *J* 6.8 Hz, 27-CH_3 or 26-CH_3), 0.74 (3H, s, 18-CH_3). Anal. Calcd. for $C_{32}H_{44}O_3$: C, 80.63; H, 9.30. Found: C, 80.7, H, 9.3.

2.3. Synthesis of 2,5,7,22-ergostatetraene 10 from the urazole adduct 9

2.3.1. Synthesis by lithium aluminum hydride reduction

To a solution of 3′,5′-dioxo-4′-phenyl-5,8[1′,2′]-1′,2′,4′-triazolidino-5α,8α-ergosta-2,6,22-triene **9** (Anastasia et al., 1985) (500 mg; 0.9 mmol) in dry tetrahydrofuran (25 ml), lithium aluminum hydride (340 mg) was added with stirring and the mixture was refluxed for 3 h under argon (Barton et al., 1971). Ethyl acetate (0.2 ml) was then added to the mixture, followed by water (7.0 ml) at 0°C. The mixture was then decanted and the solid residue dissolved into a saturated solution of sodium and potassium tartrate (10 ml) and extracted with ethyl acetate (20 ml). The organic layer collected were worked up to afford a residue which was crystallised from methanol to give the tetraene **10** (277 mg; 81%), homogeneous on TLC (plates containing 10% $AgNO_3$; eluent hexane–ethyl acetate; 90:10, v/v): m.p. 71–73°C (from methanol); $[\alpha]_D^{20}$ −3.4 (*c* 1, $CHCl_3$); UV λ_{max} 276 (ε 9400), 286 (ε 10 400), 298 nm (ε 6700); ^{1}H NMR δ 5.65–5.60 (2H, AB system, H-2 and H-3), 5.56 (1H, ddd, *J* 5.6, <1 and <1 Hz, H-6), 5.36 (1H, ddd, *J* 5.6, 2.8 and 2.8 Hz, H-7), 5.23–5.14 (2H, overlapping, H-22 and H-23), 3.00 (1H, ddd, *J* 22.0, <1 and <1 Hz, H-4a), 2.77 (1H, ddd, *J* 22.0, <1 and <1 Hz, H-4b), 1.02 (3H, d, *J* 6.6 Hz, 21-CH_3), 1.00 (3H, s, 19-CH_3), 0.90 (3H, d, *J* 6.8 Hz, 28-CH_3), 0.82 (3H, d, *J* 7.0 Hz, 26-CH_3 or 27-CH_3), 0.80 (3H, d, *J* 7.0 Hz, 27-CH_3 or 26-CH_3), 0.61 (3H, s, 18-CH_3). Anal. Calcd. for $C_{28}H_{42}$: C, 88.82; H, 11.18. Found: C, 89.0, H, 11.2.

2.3.2. Synthesis by reflux with sym-collidine

A solution of the adduct **9** (500 mg; 0.9 mmol) in *sym*-collidine (5 ml), was heated at reflux for 15 min under argon (Anastasia and Derossi, 1979). The solution was then cooled at room tempera-

ture and diluted with an ice cold aqueous solution of HCl (2 M; 20 ml). The resulting suspension was then extracted with ethyl acetate and the organic layer was worked up to afford a residue which was crystallised from methanol to give the tetraene **10** (236 mg; 69%), homogeneous on TLC (plates coated with 10% $AgNO_3$; eluent hexane–ethyl acetate; 90:10, v/v): m.p. 71–73°C (from methanol); $[\alpha]_D^{20}$ −4.1 (*c* 1, $CHCl_3$); with spectroscopic properties identical with those of the compound described above.

2.4. Synthesis of 5α,8α-ethanoergosta-2,6,22-triene-1′β,2′β-dicarboxylic acid anhydride 6

Ergosta-2,5,7,22-tetraene **10** (5.0 g; 13.2 mmol) was dissolved in xylene (50 ml) and treated with maleic anhydride (1.35 g; 13.8 mmol) under argon at 135°C for 6 h. Then the mixture was cooled, poured into ice cold water and extracted. After usual work-up, the crude residue obtained was chromatographed (eluting with hexane–ethyl acetate; 95:5, v/v) to afford the anhydride **6** (4.20 g; *Y* 67%) m.p. 118–119°C; $[\alpha]_D^{20}$ +0.18 ($CHCl_3$, *c* 1); with spectroscopic properties identical with those of the compound described above.

2.5. Reduction of 5α,8α-ethanoergosta-2,6,22-triene-1′β,2′β-dicarboxylic acid anhydride 6

2.5.1. Reduction with sodium borohydride

The anhydride **6** (300 mg; 0.63 mmol), dissolved in a solution of tetrahydrofuran–methanol (8 ml; 1:1, v/v), was treated with $NaBH_4$ (23.3 mg; 0.62 mmol) under stirring at room temperature for 12 h. At this time, the mixture was treated with water (5 ml) and extracted with ethyl acetate (15 ml). Usual work-up and rapid chromatography (eluting with hexane-dichloromethane; 50:50, v/v) afforded the 5α,8α-ethanoergosta-2,6,22-triene-1′β-hydroxymethylene-2′β-carboxylic acid lactone **3** (Allevi et al., 1999) (57 mg; *Y* 20%) as a white solid: m.p. 132–133°C; $[\alpha]_D^{20}$ −131.7; ^{1}H NMR δ 6.26 (1H, d, *J* 9.1, H-7), 5.82 (1H, d, *J* 9.1, 6-H), 5.68 (1H, m, H-3), 5.57 (1H, m, H-2), 5.22-5.14 (2H, overlapping, H-22 and H-23), 4.05 (1H, dd, *J* 9.2 and 9.2, H-4′β), 3.78 (1H, dd, *J* 9.2 and 6.0, H-4′α), 3.04 (1H, ddd, *J* 9.7, 9.2 and 6.0, H-1′), 2.53 (1H, dddd, *J* 12.5, 9.8, 7.9 and 3.5, H-15α), 2.42 (1H, d, *J* 9.7, H-2′), 1.00 (3H, d, *J* 6.8 Hz, 21-CH_3), 0.90 (3H, d, *J* 6.8 Hz, 28-CH_3), 0.83 (3H, d, *J* 7.0 Hz, 26-CH_3 or 27-CH_3), 0.81 (3H, d, *J* 7.0 Hz, 27-CH_3 or 26-CH_3), 0.79 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3).

Further elution yielded 5α,8α-ethanoergosta-2,6,22-triene-2′β-hydroxymethylene-1′β-carboxylic acid lactone **4** (Allevi et al., 1999) (178 mg; *Y* 61%) as a white solid: m.p. 120–122°C; $[\alpha]_D^{20}$ +58.3; ^{1}H NMR δ 6.19 (1H, d, *J* 9.1, H-7), 5.89 (1H, d, *J* 9.1, H-6), 5.72 (1H, m, H-3), 5.63 (1H, m, H-2), 5.21 (1H, dd, *J* 15.0 and 7.5, H-22 or H-23), 5.14 (1H, dd, *J* 15.0 and 8.0, H-23 or H-22), 4.12 (1H, dd, *J* 9.1 and 9.1, H-3′β), 3.76 (1H, dd, *J* 9.1 and 5.8, H-3′α), 3.21 (1H, dd, *J* 18.0 and 6.1, H-4α), 2.77 (1H, d, *J* 9.5, H-1′), 2.52 (1H, ddd, *J* 9.5, 9.1 and 5.8, H-2′), 0.99 (3H, d, *J* 6.8 Hz, 21-CH_3), 0.90 (3H, d, *J* 6.8 Hz, 28-CH_3), 0.82 (3H, d, *J* 7.0 Hz, 26-CH_3 or 27-CH_3), 0.80 (3H, d, *J* 7.0 Hz, 27-CH_3 or 26-CH_3), 0.79 (3H, s, 19-CH_3), 0.77 (3H, s, 18-CH_3).

2.5.2. Reduction with lithium borohydride

Treatment of anhydride **6** (200 mg; 0.42 mmol) with $LiBH_4$ in the conditions described above for the reduction with $NaBH_4$, afforded the lactone **3** (50 mg; *Y* 26%) and lactone **4** (109 mg; *Y* 56%).

2.5.3. Reduction with L-selectride

The anhydride **6** (225 mg; 0.47 mmol) was dissolved in dry, freshly distilled THF (15 ml) at 25°C under argon. L-Selectride (1.4 ml of a 1 M solution in THF; 1.4 mmol) was then injected slowly and the reaction mixture was stirred for 5 h at 25°C. At this time NaOH (0.7 ml of an aqueous 4 M solution) and H_2O_2 (1 ml of a 30% solution) were added and the stirring was continued overnight. The reaction was then acidified with an ice cold aqueous solution of HCl (2 M) and extracted with ethyl acetate. Usual work-up and rapid chromatography (eluting with hexane–dichloromethane; 50:50, v/v) afforded lactone **3** (116 mg; *Y* 53%) and lactone **4** (60 mg; *Y* 28%) identical in all aspects with the compounds above described.

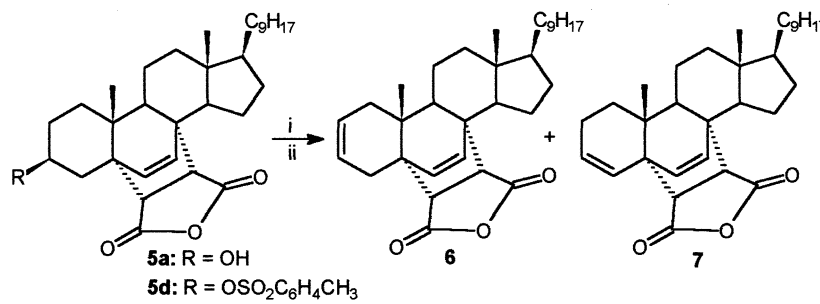

Fig. 2. Schematic pathway of the synthesis of Δ^2-unsaturated anhydride **6** via dehydration of Inhoffen adduct. Reagents and reaction conditions: (i) *p*-toluenesulfonyl chloride, pyridine, 23°C, 20 h; (ii) *sym*-collidine, reflux, 8 h.

2.5.4. Reduction with K-selectride

Treatment of anhydride **6** (225 mg; 0.47 mmol) with K-selectride in the conditions described above for the reduction with L-selectride, afforded the lactone **3** (111 mg; *Y* 51%) and lactone **4** (54 mg; *Y* 25%).

Unvaried results were obtained performing the reductions with $NaBH_4$ or $LiBH_4$ at 0°C and at -10°C and the reductions with L- and K-selectride at -40°C.

3. Results and discussion

Earlier work (Allevi et al., 1997; Rickard and Le Quesne, 1998) on the metal hydride reduction of the cyclic anhydrides **5a** and **b** suggested that the 3β-substituents influence the regiochemistry of the reductions of the anhydride group. In fact, the 3β-hydroxy and the 3β-acetoxy substituents direct the reduction completely to the carbonyl nearest to the ring A (Allevi et al., 1997; Rickard and Le Quesne, 1998) while the 3β-methyl (Burke and Le Quesne, 1971) and 3β-thexyldimethylsilyloxy substituents (Allevi et al., 1999) show lower directing effect and permit the obtaining of both possible lactones in roughly equal amounts, almost using sodium borohydride. Because of these results we considered that the Δ^2 anhydride **6** (Fig. 2), lacking of any substituent at the 3β-position of the steroid nucleus, could be a suitable precursor of the unsaturated lactone **4**. In fact we were confident that the absence of any 3β-substituent eliminates its unfavourable influence on the regioselectivity of the hydride reduction and could decrease the steric hindrance at the 4′-carbonyl which is believed one of the factors which favour its reduction (Soucy et al., 1987 and references therein).

With this in mind we decided to prepare the anhydride **6** starting from the Inhoffen adduct **5a** (Fig. 2) which was transformed into the corresponding Δ^2-unsaturated compound **6** via dehydrotosylation of the corresponding 3β-*p*-toluensulfonate **5d**, by treatment with *sym*-collidine at reflux. These conditions preserved the anhydride group but promote a non regiospecific dehydrotosylation which affords the compound **6** (in 59% yield; 7% total yield from ergosterol) always accompanied by considerable amounts of the Δ^3-isomer **7** (32%) unsuitable for the synthesis.

The formation of the undesired Δ^3-anhydride was quite unexpected considering our previous result in the dehydration of the Diels–Alder adduct of ergosterol **8** which was transformed exclusively into the Δ^2-compound **9** by a similar reaction sequence (Anastasia et al., 1985). On the other hand, the obtaining of the Δ^2-compound **9** from ergosterol (in 65% yield) suggested that the anhydride **6** could be prepared from ergosterol via the adduct **9** (Fig. 3). In fact this compound contains a masked $\Delta^{5,7}$ system which could be regenerated (Barton et al., 1971; Anastasia and Derossi, 1979; Anastasia et al., 1985) in a reaction giving the triene **10** able to react with maleic anhydride to afford the anhydride **6**. Thus, the treatment of the adduct **9** with $LiAlH_4$ (Barton et al., 1971) or *sym*-collidine (Anastasia and

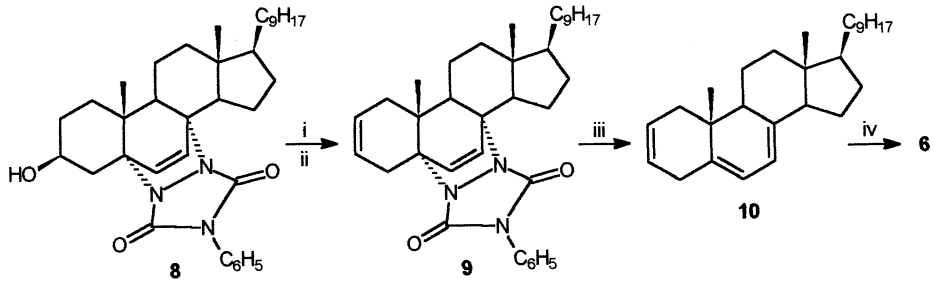

Fig. 3. Schematic pathway of the synthesis of Δ^2-unsaturated anhydride **6** via tetraene **10**. Reagents and reaction conditions: (i) *p*-toluenesulfonyl chloride, pyridine, 23°C, 21 h; (ii) basic Al_2O_3, toluene, stirring at r.t. for 10 h; (iii) $LiAlH_4$, THF, reflux, 3 h; (iv) maleic anhydride, xylene, 135°C, 6 h.

Derossi, 1979) regenerated the $\Delta^{5,7}$ dienic system typical of ergosterol affording the crystalline tetraene **10** uncontaminated by the Δ^3-isomer. This tetraene was then reacted with maleic anhydride in a Diels–Alder reaction affording the anhydride **6** in satisfactory yields (67% yield).

The anhydride **6** was then subjected to reduction with different hydride (sodium and lithium borohydride, L- and K-selectride (Makhlouf and Rickborn, 1981) at different temperatures. The obtained results showed that, in all cases, an incomplete regioselectivity of the reduction occurred since both possible lactones **3** and **4** were formed. However while L- and K-selectride afforded as major compound the undesired lactone **3**, $NaBH_4$ and $LiBH_4$ afforded the lactone **4** as major component (56–61%) which contained the lactone **3** in minor ratio (20–26%), independently from the temperature.

Therefore, the lactone **4** was prepared using $NaBH_4$ and transformed into the brassinolide analogue **2** according to the previously reported procedure (Allevi et al., 1999). Thus, the improvement of its preparation by a different route represents an improvement of the synthesis of lactone **2**.

In conclusion, we report here an improved method for converting inexpensive ergosterol to lactone **4** (in 21% total yield) and consequently to the brassinolide analogue **2** which is now easily obtainable in amounts suitable for biological experiments. Moreover, the results achieved in the reductions of the cyclic anhydride **6** together with those previously reported (Allevi et al., 1997, 1999; Rickard and Le Quesne, 1998) show that the presence and the nature of a 3β-substituent in the Inhoffen anhydrides are dominant factors in influencing the course of the metal hydride reductions of Inhoffen adducts. In their absence steric effects prevail and favour the reduction of the more hindered carbonyl (Bloomfield and Lee, 1967). In this case, the nucleophile's reactivity and the effect of the counterions of the hydride reagents appear on the contrary negligible (Bloomfield and Lee, 1967).

Acknowledgements

This work was supported by Ministero dell' Università e della Ricerca Scientifica e Tecnologica (MURST; fondi 60%).

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