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Synthesis of new Brassinosteroids with Epoxy Functions: the Effect on the Regioselectivity of the Baeyer-Villiger Reaction

Carme Brosa* and Xavier Miró

Department of Organic Chemistry, CETS Institut Químic de Sarrià,

Universitat Ramon Llull E-08017 Barcelona, Spain.

Abstract: The synthesis of 7 new brassinosteroid analogs having epoxy function in A ring and side chain together with ketone or lactone in B ring is reported. The epoxidation and regioselective lactonization of the diene **3** have been achieved in only one step using molecular oxygen and aldehyde. The influence of either an epoxide or a double bond at C2,C3 on the regioselectivity of the Baeyer-Villiger reaction is discussed. © 1997 Elsevier Science Ltd.

The interest in brassinosteroids lies on their role as stress and pathogenic disease resistance in plants as well as plant growth regulators and promoters.[1,2]

Much synthetic effort has been done not only to find brassinosteroids with high activity for further application in agriculture but also to delve into their knowledge from the structural, physiological and biogenetic points of view. In this sense, several new analogs have been synthesized and promising results have been obtained.[3,4] However it must be mentioned that laboratory biotests do not give a complete idea of the growth capacity of brassinosteroids when they were applied in the field. One example of this is found with the three analogs with a 22,23 epoxide on side chain ((22R,23R)-2α,3α-isopropylidenedioxy-22,23-epoxy-7-oxa-B-homo-5α-stigmastan-6-one, (22R,23R)-2α,3α-diacetoxy-22,23-epoxy-7-oxa-B-homo-5α-ergostan-6-one and (22S,23S)-2α,3α-diacetoxy-22,23-epoxy-7-oxa-B-homo-5α-ergostan-6-one) (Figure 1) that did not exhibit activity in the rice lamina inclination test but, under field conditions, a greater increase in yield was obtained as compared to brassinolide.[5,6]

This suggests that the epoxy function in side chain may be the biosynthetic precursor of the corresponding diol. Moreover, brassinosteroids with this kind of functionality are very useful for our QSAR studies.[3,7]

With these points in mind we report the synthesis of new brassinosteroid analogs having two epoxy functionalities in A ring and side chain, together with lactone or ketone in B ring.

Our target molecules are the compounds **1** and **2** (Figure 2).

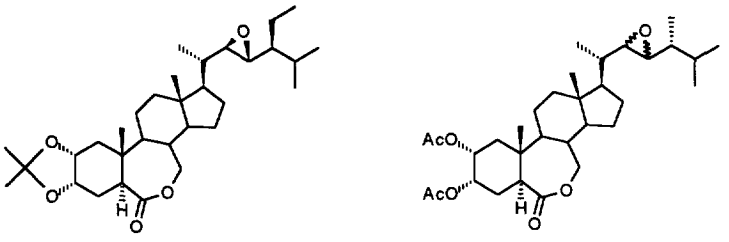
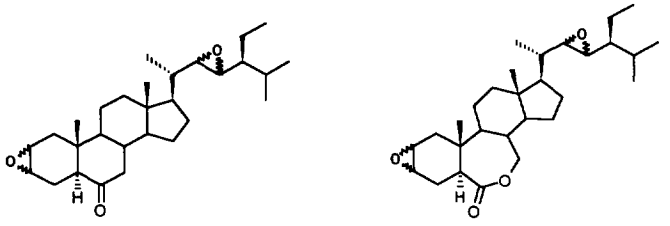


Figure 1

Beside the potential role as biogenetic precursors, their activity evaluation and molecular mechanic calculations will allow in depth knowledge of the structural requirements needed for a brassinosteroid to be active.

The aim of this study is to evaluate the possibility of obtaining these target molecules in a high regioselectivity through a synthetic route including the least number of steps. In this paper we present the results obtained using two types of reagents: i) *m*-chloroperbenzoic acid (MCPBA) which is known to be adequate to obtain epoxides from double bonds as well as lactones from ketones by Baeyer-Villiger reaction,[8] and ii) molecular oxygen and an aldehyde suitable to obtain both epoxides and lactones but that has never been studied to produce both transformations at the same time.[9-13]

Also, the influence of the epoxy group and double bond at A ring on the regioselectivity in the Baeyer-Villiger oxidation will be discussed.



1 2

Figure 2

Results

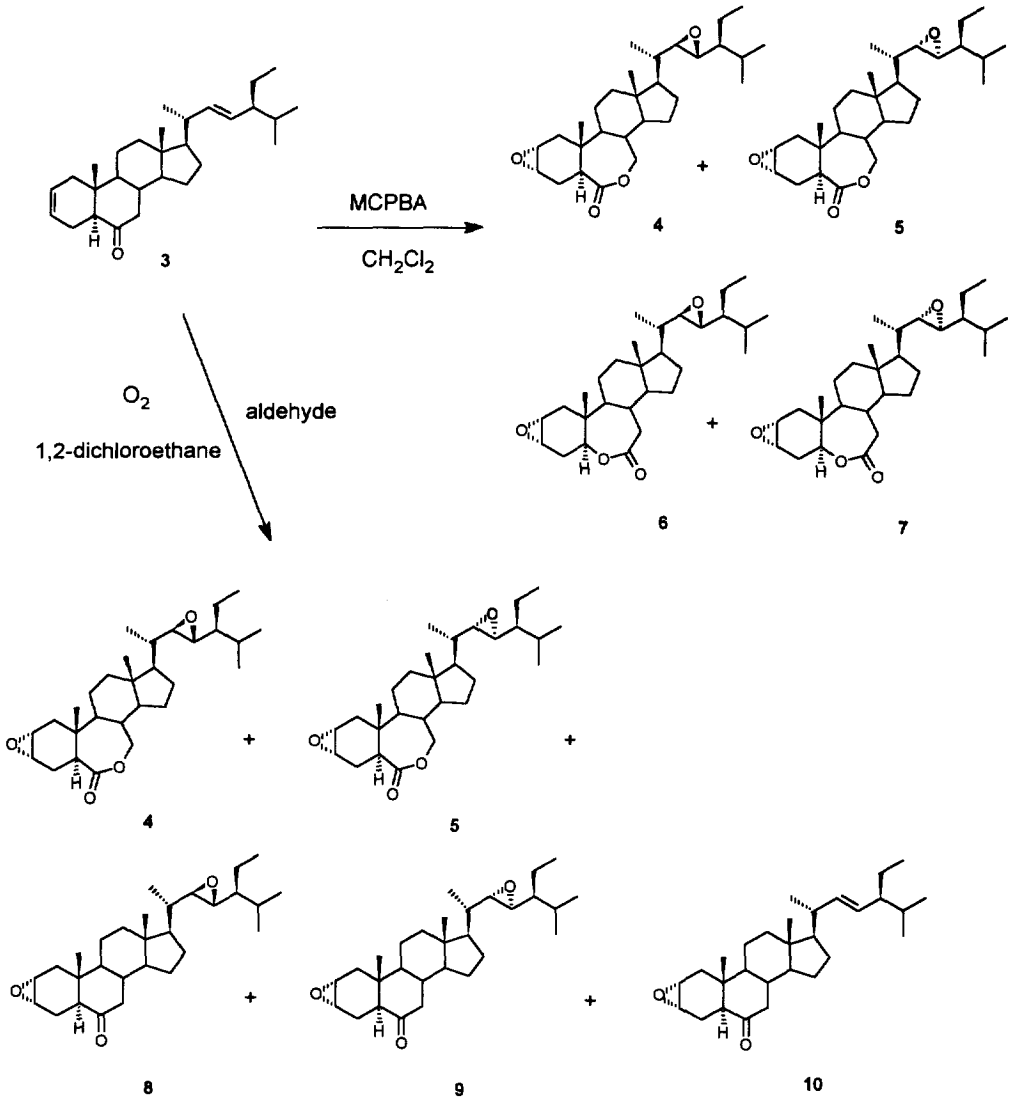
The diene **3** was obtained starting from stigmasterol following the strategy previously described.[4]

Treatment of the diene **3** with MCPBA (3.2 eq.) at 0°C for 29 h yielded a complex mixture which, after successive vacuum chromatographies, allowed the isolation and identification of the diastereoisomeric diepoxides **4** and **5**, both with 7-oxa-lactone function, and the diastereoisomeric diepoxides **6** and **7**, both with 6-oxa-lactone function (Scheme 1). The diastereoisomeric ratio in both cases was 2.2:1 and 2.1:1 respectively.

The use of molecular oxygen in the presence of an aldehyde has been shown to be useful for a Baeyer-Villiger oxidation and epoxidation separately. Nevertheless, the type of aldehyde seems to play an important role in obtaining a good yield in these transformations. In the oxidation of **3** we have examined isobutyraldehyde, one of the best reagents for epoxidation of double bonds,[10] and benzaldehyde, which is suitable for ketone lactonization.[11] The latter has also been evaluated in the presence of $Cu(AcO)_2$.[10]

The general procedure consist of bubbling oxygen into a solution of **3**, the aldehyde (30 eq.) and $Cu(AcO)_2$ (0.03 eq.), if added, in 1,2-dichloroethane at 40°C.

A thin layer chromatography (TLC) monitorization of a preliminar assay enabled us to identify the compounds **4** and **5**. No compounds with 6-oxa-lactone function (**6** and **7**) were detected. Also, few compounds with a polarity between the starting diene **3** and the lactones **4** and **5** were observed. To identify these compounds, a reaction was performed which, after column chromatography and preparative TLC, allowed us to isolate and identify the diepoxyketones **8** and **9** and the monoepoxyketone **10** (Scheme 1).



Scheme 1

The monitorization of all these reactions by HPLC showed that the main products (**4** and **5**) were obtained in a range of 41-49% and 19-33% respectively, depending on the aldehyde used and the presence or absence of $Cu(AcO)_2$. The so obtained results at different timepoints for all compounds are represented in Figure 2. It can be seen that the amount of the starting diene **3** decreased as the main products **4** and **5** increased. However, the amount of **10**, **8** and **9** remained unchanged throughout the reaction suggesting that they are intermediates of the final lactones **4** and **5**. With respect to the diastereoselectivity, the ratio 22R,23R/22S,23S found in all cases was similar to the compounds obtained with MCPBA. Also, the different aldehydes as well as the addition of $Cu(AcO)_2$ did not seem to have a strong influence on the results obtained.

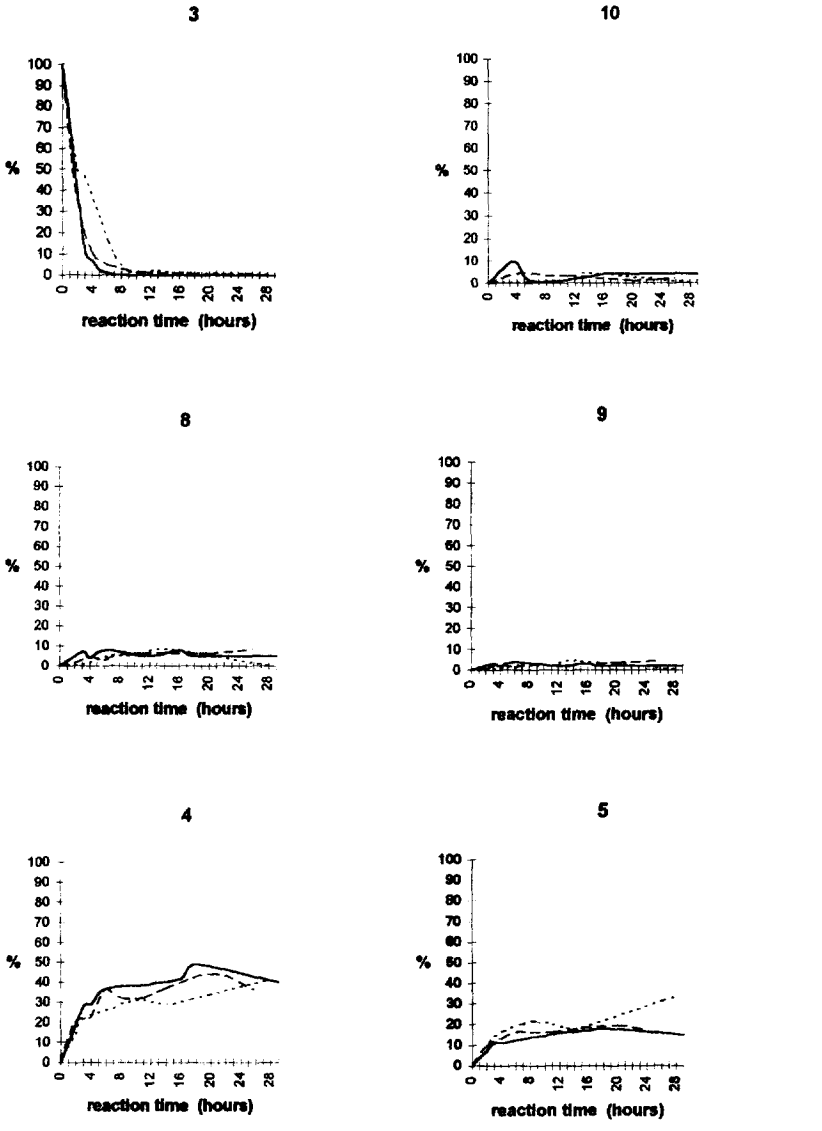
Some conclusions can be drawn by comparing the results obtained with MCPBA and those obtained with molecular oxygen and aldehyde. The epoxidation of the double bond Δ^2 took place in all cases by attack on the less hindered site giving stereoselectively the 2α,3α-epoxide. Nevertheless, the epoxidation of the Δ^{22} double bond furnished a diasteroisomeric mixture (R,R and S,S) due to the presence of the alkyl substituent at C24.

On the other hand, the fact of isolating compounds **8**, **9** and **10** without lactone function in B ring using molecular oxygen and aldehyde, not detected using MCPBA, indicates that the epoxidation of the double bonds took place before the lactonization of the ketone at C6. Also, the isolation of **10** but not the corresponding monoepoxide at C22-C23 indicates that the epoxidation of Δ^2 took place before those of Δ^{22} as expected due to the hindrance produced by the alkyl substituent at C24. Therefore, using molecular oxygen and aldehyde the following reactivity order for the different functionalities present at **3** could be established : $\Delta^2 > \Delta^{22} >$ 6-ketone.

Another interesting point that should be emphasized is that, using MCPBA as reagent, both 6-oxa (**6** , **7**) and 7-oxa (**4** , **5**) lactones were isolated, whereas only 7-oxa lactones (**4** , **5**) were isolated when molecular oxygen was used. Taking into account the mechanism of Baeyer-Villiger oxidation on 6-keto steroids, the reaction yielded the 6-oxa or 7-oxa lactone depending on the feasibility of the migration of C5 or C7 carbons, respectively. It has been demonstrated that the presence of some electron-withdrawing groups such as hydroxyl, acyloxyl or sulfonyloxyl in A ring favour the migration of C7 carbon yielding the 7-oxalactone[14] but this effect had never been evaluated in the presence of epoxides or double bonds. According to the products that we have obtained when molecular oxygen and aldehyde are used, and assuming that epoxidation takes place before lactonization, the high regioselectivity induced in the 7-oxa-lactones indicates that the previously formed 2α,3α-epoxy function would have enough of an inductive effect to direct the migration of C7, yielding the 7-oxa-lactones regioselectivity. Nevertheless, since both regioisomers occur in MCPBA, because both regioisomers occur, we can assume that the epoxidation does not take place prior to lactonization. Therefore, 6-oxa-lactone formation is necessarily due to the presence of the double bond $\Delta^{2.}$.

In conclusion, the results obtained using MCPBA or molecular oxygen and aldehyde allow to assume that both procedures are able to epoxidize the double bonds and to lactonize the ketone, although in a different way. With molecular oxygen the epoxidation occurs prior to the lactonization. Moreover, the use of this reagent has evidenced that an epoxide function in A ring is enough to regioselectively direct the lactonization to the 7-oxa-lactone whereas the double bond is not.

Furthermore, starting from the diene **3** and in one step only we have synthesized 7 new brassinosteroid analogs having epoxy functionalities in A ring and in side chain, and 6-ketone, 6-oxa-lactone or 7-oxa-lactone in B ring. Neither of them has exhibited activity in the standard rice lamina inclination test, that is, after 2 days. However, when the bioassay was followed up to 7 days, we detected a progressive activity increase. This effect was not observed with the reference 24-epibrassinolide. In our opinion, this result, together with the recent isolation of a natural brassinosteroid with epoxy function in A ring (secasterone)[15] from *Secale cereale*, supports the potential role of the epoxy function as biogenetic precursor of the diols present in brassinosteroids on A ring and side chain. The results obtained in the evaluation of the activity of such compounds will soon be published. Also their molecular mechanic calculation are currently in progress.



____ : isobutyraldehyde ------ : benzaldehyde _ _ _ : benzaldehyde, $Cu(AcO)_2$

Figure 2

Experimental Section

Melting points were determined on a Gallenkamp instrument and are uncorrected. IR spectra were obtained on a Perkin-Elmer 683 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian-Germini-300 (300 MHz) spectrometer, using TMS as internal standard. The multiplicity of the signals in the ^{13}C-NMR spectra was determined using the sequence Distorsionless Enhancement Polarization Transfer (D.E.P.T). Mass spectra (electron impact, (EIMS) m/z) were run on a Hewlett-Packard 5995-A spectrometer and mass spectra (chemical ionization, (CIMS) m/z) were run on a Hewlett-Packard 5988-A using methane as the carring gas. The progress of all reactions and column chromatography were monitored by TLC on silica gel $60F_{254}$ microplates (Macherey-Nagel, Art 804023) and spots were detected by spraying 50% sulfuric acid, followed by heating (125°C). Column chromatography was performed on silica gel 63-200 μm (70-230 mesh), vacuum column chromatography on silica gel 15 μm, and medium-pressure chromatograpy on a Lichoprep Si 60 (230-400 mesh) (Merck). "Usual work-up" refers to dilution with water, extraction with an organic solvent, washing the extract to neutrality, drying ($MgSO_4$) and removal of the solvent under reduced pressure. The composition of the reaction crudes were determined by high-performance liquid chromatography (HPLC) using a Waters 600E chromatograph, an ultraviolet Waters (λ=210 nm) detector, a Waters Novapack RP-18 (ϕ_i = 3.9 mm), μ = 4 μm, L = 15 m) column and CH_3CN / H_2O 9:1 as mobil phase.

MCPBA treatment of 3. 5g (15.9 mmol) of MCPBA (55%) was added to a solution of 2.000g (4.88 mmol) of (22E)-5α-stigmasta-2,22-dien-6-one (**3**)[4] in 150 mL of CH_2Cl_2 at 0°C and stirred at this temperature for 29 h. The reaction mixture was filtered to eliminate the *m*-chlorobenzoic acid formed and 40 mL of Na_2SO_3 were added and stirred for 5 h. The work-up (AcOEt) yielded 2.12g of a white solid, wich was purified by medium pressure chromatography (Cy/AcOEt 7:1) to give (22R,23R) and (22S,23S)-2α,3α,22,23-diepoxy-5α-stigmasta-6,7-seco-6,7-lactone (**4**, **5**) (94 mg, 13%, 2.2:1 measured by H.P.L.C.), and (22R,23R) and (22S,23S)-2α,3α,22,23-diepoxy-5α-estigmasta-5,6-seco-6,5-lactone (**6**, **7**) (118 mg, 16%, 2.1:1 measured by HPLC). Purification of these mixtures by vacuum chromatography ($CHCl_3$/acetone 35:1) yielded (22R,23R)-2α,3α,22,23-diepoxy-5α-stigmasta-6,7-seco-6,7-lactone (**4**) (18 mg, 3.4 %), (22S,23S)-2α,3α,22,23-diepoxy-5α-stigmasta-6,7-seco-6,7-lactone (**5**) (12 mg, 2.3 %), (22R,23R)-2α,3α,22,23-diepoxy-5α-stigmasta-5,6-seco-6,5-lactone (**6**) (34 mg, 6.4 %), (22S,23S)-2α,3α,22,23-diepoxy-5α-stigmasta-5,6-seco-6,5-lactone (**7**) (8 mg, 1.5 %).

(22R,23R)-2α,3α,22,23-diepoxy-5α-stigmasta-6,7-seco-6,7-lactone (**4**): m.p. 104-107°C; IR ν^{CHCl_3} cm^{-1}: 2960, 2860, 1735, 1465, 1340, 1320, 1275, 1220, 1180, 905, 820; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 4.04 (2H, m, $W_{1/2}$=18 Hz, H-C-7), 3.32 (1H, m, $W_{1/2}$=1.8 Hz, βH-C-3), 3.16 (1H, m, $W_{1/2}$=4.2 Hz, βH-C-2), 3.11 (1H, dd, J=4.8 Hz, J=11.6 Hz, αH-C-5), 2.73 (1H, dd, J=2.4 Hz, J=7.2 Hz, H-C-22), 2.49 (1H, m, $W_{1/2}$=3 Hz, H-C-23), 1.03-0.91 (15H, m, 19, 21, 26, 27, 29-CH_3), 0.70 (3H, s, 18-CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) : δ 175.9 (s, C-6), 70.2 (t, C-7), 61.9 (d, C-22), 61.8 (d, C-23), 59.5 (d) + 51.2 (d) (C-9 and C-14), 53.3 (d, C-17), 52.0 (d, C-3), 50.5 (d, C-2), 48.3 (d, C-24), 43.0 (s, C-13), 41.3 (d, C-5), 40.0 (d, C-8), 39.9 (t, C-1), 39.5 (t, C-12), 38.5 (d, C-20), 34.0 (s, C-10), 29.1 (d, C-25), 27.6 (t, C-4), 25.2 (t, C-16), 24.9 (t, C-15), 22.6 (t, C-11), 20.9 (t, C-28), 20.1 (q, C-26), 19.6 (q, C-27), 18.0 (q, C-19), 15.9 (q, C-29), 12.4 (q, C-21), 11.9 (q, C-18); CIMS (h.r.) m/z: 459.3445 $[M+1]^+$ (calc. 459.3474).

(22S,23S)-2α,3α,22,23-diepoxy-5α-stigmasta-6,7-seco-6,7-lactone (**5**): m.p. 159-163°C; IR ν^{CHCl_3} cm^{-1}: 2990, 2910, 1750, 1490, 1340, 1245, 1200, 930, 845; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 4.04 (2H, m, $W_{1/2}$=20 Hz, H-C-7), 3.32 (1H, m, $W_{1/2}$=1.8 Hz, βH-C-3), 3.15 (1H, m, $W_{1/2}$=3.9 Hz, βH-C-2), 3.10 (1H, dd, J=4.8 Hz, J=11.6 Hz, αH-C-5), 2.52-2.47 (2H, m, H-C-22, H-C-23), 1.02-0.90 (15H, m, 19, 21, 26, 27, 29-CH_3), 0.69 (3H, s, 18-CH_3); ^{13}C-RMN (75 MHz, $CDCl_3$): 175.9 (s, C-6), 70.2 (t, C-7), 62.9 (d, C-22), 59.6 (d) + 51.2 (d) (C-14 and C-9), 58.6 (d, C-23), 55.9 (d, C-17), 52.1 (d, C-3), 50.5 (d, C-2), 48.7 (d, C-24), 43.0 (s, C-13), 41.3 (d, C-5), 40.0 (d, C-8), 39.9 (t, C-1), 39.6 (t, C-12), 38.9 (d, C-20), 34.0 (s, C-10), 29.3 (d, C-25), 26.8 (t, C-4), 25.3 (t, C-16), 24.9 (t, C-15), 22.6 (t, C-11), 21.0 (t, C-28), 19.4 (q, C-26), 19.4 (q, C-27), 18.0 (q, C-19), 16.3 (q, C-29), 12.4 (q, C-21), 12.0 (q, C-18); CIMS (h.r.) m/z: 459.3490 $[M+1]^+$ (calc. 459.3474).

(22R,23R)-2α,3α,22,23-diepoxy-5α-stigmasta-5,6-seco-6,5-lactone (**6**): m.p. 121-124°C; IR ν^{CHCl_3} cm^{-1}: 2950, 2870, 1735, 1465, 1380, 1330, 1300, 1270, 1250, 1030, 980, 895, 820, 745; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 4.48 (1H, m, $W_{1/2}$=8.0 Hz, αH-C-5), 3.34 (1H, m, $W_{1/2}$=2.1 Hz, βH-C-3), 3.13 (1H, m, $W_{1/2}$=4.4 Hz, βH-C-2), 2.74 (1H, dd, J=7.1 Hz, J=2.3 Hz, H-C-22), 2.52-2.34 (4H, m, H-C-7, H-C-23, ξH-C-4), 1.03-0.92 (15H, m, 19, 21, 26, 27, 29-CH_3), 0.69 (3H, s, 18-CH_3); ^{13}C-RMN (75 MHz, $CDCl_3$): 174.6 (s, C-6), 78.9 (d, C-5), 61.9 (d, C-22), 61.7 (d, C-23), 59.0 (d, C-14), 55.1 (d, C-9), 53.5 (d, C-17), 53.3 (d, C-3), 49.9 (d, C-2), 48.2 (d, C-24), 42.9 (s, C-13), 39.7 (t, C-1), 39.4 (t, C-12), 38.4 (d, C-20), 37.9 (t, C-7), 37.6 (s, C-10), 35.2 (d, C-8), 29.1 (d, C-25), 28.8 (t, C-4), 27.2 (t, C-16), 25.4 (t, C-15), 22.4 (t, C-11), 20.8 (t, C-28), 20.1 (q, C-26), 19.6 (q, C-27), 15.9 (q, C-29), 13.9 (q, C-19), 12.4 (q, C-21), 11.7 (q, C-18); CIMS (h.r.) m/z: 459.3456 $[M+1]^+$ (calc. 459.3474).

(22S,23S)-2α,3α,22,23-diepoxy-5α-stigmasta-5,6-seco-6,5-lactone (7): m.p. 131-134°C; IR ν^{CHCl_3} cm^{-1}: 2960, 2880, 1730, 1465, 1380, 1340, 1280, 1260, 1030, 910, 825; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 4.48 (1H, m, $W_{1/2}$=8.0 Hz, αH-C-5), 3.34 (1H, m, $W_{1/2}$=2.1 Hz, βH-C-3), 3.13 (1H, m, $W_{1/2}$=4.6 Hz, βH-C-2), 2.52-2.33 (5H, m, H-C-22, H-C-23, H-C-7, ξH-C-4), 1.01-0.90 (15H, m, 19, 21, 26, 27, 29-CH_3), 0.68 (3H, s, 18-CH_3); ^{13}C-RMN (75 MHz, $CDCl_3$): 174.6 (s, C-6), 78.9 (d, C-5), 62.8 (d, C-22), 59.1 (d, C-14), 58.5 (d, C-23), 56.2 (d, C-17), 55.1 (d, C-9), 53.4 (d, C-3), 49.9 (d, C-2), 48.7 (d, C-24), 42.9 (s, C-13), 39.7 (t, C-1), 39.5 (t, C-12), 38.8 (d, C-20), 38.0 (t, C-7), 37.6 (s, C-10), 35.3 (d, C-8), 29.3 (d, C-25), 28.9 (t, C-4), 26.4 (t, C-16), 25.4 (t, C-15), 22.5 (t, C-11), 21.0 (t, C-28), 19.4 (q, C-26), 19.4 (q, C-27), 16.2 (q, C-29), 14.0 (q, C-19), 12.4 (q, C-21), 11.9 (q, C-18); CIMS (h.r.) m/z: 4593506 $[M+1]^+$ (calc. 459.3474).

Molecular oxygen and aldehydes treatment of 3.

Preliminar reaction. Oxygen was bubbled at 40 °C in a solution of 3.3 mmol of isobutyraldehyde in 1,2-dichloroethane for 45 minutes. 100 mg (0.24 mmol) of **3** dissolved in 1 mL of 1,2-dichloroethane was added, and the resulting mixture was stirred with bubling oxygen at 40 °C for 17 h. After addition of 20 mL of Na_2SO_3 (5%) and stirring for 20 minutes, the work-up afforded a crude, which was purified first by chromatography ($CHCl_3$/Hex 3:1, $CHCl_3$ and $CHCl_3$/acetone 35:1) to give 6 mg of (22E)-2α,3α-epoxy-5α-stigmast-22-en-6-one (**10**), and 17 mg of a mixture, which was purified by preparative thin layer chromatography ($CHCl_3$/Acetone 35:1) to give 1 mg of (22R,23R)-2α,3α,22,23-diepoxy-5α-stigmast-6-one (**8**) (Rf = 0.33) and 2 mg of (22S,23S)-2α,3α,22,23-diepoxy-5α-stigmast-6-one (**9**) (Rf = 0.27).

(22E)-2α,3α-epoxy-5α-stigmast-22-en-6-one (**10**) : m.p. 168-171°C; IR ν^{CHCl_3} cm^{-1}: 2960, 2880, 1715, 1465, 1435, 1390, 800, 755; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 5.14 (1H, dd, J=8.4 Hz, J=15 Hz, H-C-22), 5.02 (1H, dd, J=8.4 Hz, J=15.1 Hz, H-C-23), 3.27 (1H, m, $W_{1/2}$=1.8 Hz, βH-C-3), 3.13 (1H, m, $W_{1/2}$=4.7 Hz, βH-C-2), 2.39-2.29 (2H, m, αH-C-5, βH-C-7), 0.71 (3H, s, 19-CH_3), 0.69 (3H, s, 18-CH_3); ^{13}C-RMN (75 MHz, $CDCl_3$): 211.3 (s, C-6), 62.1 (d, C-22), 61.9 (d, C-23), 56.2 (d, C-14), 53.4 (d, C-17), 53.1 (d, C-9), 52.4 (d, C-3), 50.1 (d, C-2), 49.9 (d, C-5), 46.9 (t, C-7), 42.6 (s, C-13), 40.4 (d, C-20), 39.3 (t, C-12), 38.5 (s, C-10), 37.9 (t, C-1), 37.5 (d, C-8), 31.9 (d, C-25), 28.7 (t, C-16), 25.4 (t, C-28), 24.0 (t, C-15), 21.2 (q, C-21), 21.1 (q, C-26), 21.0 (t, C-11), 21.0 (t, C-4), 19.0 (q, C-27), 15.0 (q, C-19), 12.2 (q, C-29), 12.1 (q, C-18); CIMS (h.r.) m/z: 427.3580 $[M+1]^+$ (calc. 427.3576).

(22R,23R)-2α,3α,22,23-diepoxy-5α-stigmast-6-one (**8**) : m.p. 144-148 °C; IR ν^{CHCl_3} cm^{-1}: 2960, 2870, 1715, 1460, 1380, 1260, 900, 800; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 3.27 (1H, m, $W_{1/2}$=1.9 Hz, βH-C-3), 3.12 (1H, m, $W_{1/2}$=4.7 Hz, βH-C-2), 2.74 (1H, dd, J=2.4 Hz, J=7.1 Hz, H-C-22), 2.49 (1H, dd, J=2.4 Hz, J=6.0 Hz, H-C-23), 2.40-2.30 (2H, m, αH-C-5, βH-C-7), 0.71 (3H, s, 19-CH_3), 0.66 (3H, s, 18-CH_3); ^{13}C-RMN (75 MHz, $CDCl_3$): 211.2 (s, C-6), 62.1 (d, C-22), 61.9 (d, C-23), 56.2 (d, C-14), 53.4 (d, C-17), 53.1 (d, C-9), 52.4 (d, C-3), 50.1 (d, C-2), 49.9 (d, C-5), 48.3

(d, C-24), 46.9 (t, C-7), 43.0 (s, C-13), 39.2 (t, C-12), 38.6 (d, C-20), 38.4 (s, C-10), 37.9 (t, C-1), 37.5 (d, C-8), 29.2 (d, C-25), 27.7 (t, C-16), 24.2 (t, C-15), 21.0 (t, C-4), 21.0 (t, C-11), 20.9 (t, C-28), 20.2 (q, C-26), 19.6 (q, C-27), 16.1 (q, C-29), 15.0 (q, C-19), 12.4 (q, C-21), 11.9 (q, C-18); CIMS (h.r.) m/z: 443.3586 $[M+1]^+$ (calc. 443.3525).

(22S,23S)-2α,3α,22,23-diepoxy-5α-stigmast-6-one (**9**) : m.p. 156-159 °C; IR ν^{CHCl_3} cm^{-1}: 2960, 2870, 1715, 1470, 1385, 1260, 910, 800; ^{1}H-RMN (300 MHz, $CDCl_3$): δ 3.27 (1H, m, $W_{1/2}$=1.9 Hz, βH-C-3), 3.12 (1H, m, $W_{1/2}$=4.8 Hz, βH-C-2), 2.53-2.48 (2H, m, H-C-22, H-C-23), 2.39-2.30 (2H, m, αH-C-5, βH-C-7), 0.71 (3H, s, 19-CH_3), 0.65 (3H, s, 18-CH_3); ^{13}C-RMN (75 MHz, $CDCl_3$): 211.2 (s, C-6), 62.9 (d, C-22), 58.5 (d, C-23), 56.1 (d, C-14), 55.9 (d, C-17), 53.2 (d, C-9), 52.4 (d, C-3), 50.1 (d, C-2), 49.9 (d, C-5), 48.7 (d, C-24), 46.8 (t, C-7), 43.1 (s, C-13), 39.2 (t, C-12), 38.7 (d, C-20), 38.4 (s, C-10), 37.9 (t, C-1), 37.4 (d, C-8), 29.3 (d, C-25), 26.8 (t, C-16), 24.1 (t, C-15), 21.0 (t, C-4), 21.0 (t, C-11), 20.9 (t, C-28), 19.4 (q, C-26), 19.3 (q, C-27), 16.2 (q, C-29), 15.0 (q, C-19), 12.3 (q, C-21), 12.0 (q, C-18); CIMS (h.r.) m/z: 443.3514 $[M+1]^+$ (calc. 443.3525).

Monitorization by HPLC : general procedure. A solution of 3.0 mmol of aldehyde and 5 mg (0.03 mmol) of $Cu(AcO)_2$ (if added) in 5 mL of 1,2-dichloroethane at 40 °C was bubbled with oxygen for 30 min. A solution of 40 mg (0.10 mmol) of **3** in 1 mL of 1,2-dichloroethane was added, and the resulting mixture was stirred with bubling oxygen at 40 °C for 28 h. The reaction was monitored by HPLC at different times with the following procedure : 0.1 mL of the reaction mixture was extracted with 8 mL of 1,2-dichloroethane and washed with Na_2SO_3 (5%). After usual work-up, the crude was dissolved with MeOH at 0.2 mg/mL.

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