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Oxidation of Δ^4 - and Δ^5 -Steroids with Hydrogen Peroxide Catalyzed by Porphyrin Complexes of Mn^{III} and Fe^{III}

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In this paper we describe a new environmentally friendly method to promote the stereoselective epoxidation of Δ^4 - and Δ^5 -steroids. Metalloporphyrins efficiently catalyze the epoxidation reactions of 17 β -acetoxy-4-androstene (**1**), 4-cholestene (**2**) and 3 β -acetoxy-5-cholestene (**3**) in the presence of H_2O_2 as oxygen donor. Modeling the molecular structure of the porphyrin as well as the central metal allows the control of the preferential formation of α - or β -epoxides. Porphyrins with bulky, electron-withdrawing groups in the *ortho* positions of the *meso* phenyls and with Mn^{III} as the central metal ion, such as $[\text{Mn}(\text{TDCPP})\text{Cl}]$, gave preferentially the β -epoxide of Δ^4 - and Δ^5 -steroids. $[\text{Fe}(\text{TPFP})\text{Cl}]$ catalyzes preferen-

tially the α -epoxidation of Δ^4 -steroids and also increases the stereoselectivity for the α -epoxide in Δ^5 -steroids, similar to the results obtained with *m*-CPBA (*m*-chloroperbenzoic acid) as oxidant. The substrate structure strongly influences the chemoselectivity of the reactions. The X-ray structures of two main products were determined, and two-dimensional NMR techniques allowed the full assignment of ^1H and ^{13}C NMR resonances as well as the stereochemistry of these products. A mechanistic proposal involving oxo species for the β -approach and peroxy species for the α -approach is proposed.

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Introduction

The chemical modification of steroids can bring significant biological effects.^[1] This is obviously related to the application of specific steroids as valuable therapeutic agents in the treatment of several diseases, more recently in the treatment of breast cancer.^[2] Breast-cancer growth is presumably related to the presence of estrogens;^[2] these are biosynthesized in vivo from androgens in three successive oxidative steps, mediated by aromatase, a complex enzyme of the cytochrome P450 family.^[3,4] Therefore, inhibitors of this enzyme have been developed in the last few years as an approach to a treatment strategy. Compounds like 4-hydroxyandrostane-3,17-dione and 6-methyleneandrostane-1,4-diene-3,17-dione were found to be effective as inhibitors of aromatase by blocking the estrogen biosynthesis and thereby causing tumor regression.^[2] In order to improve the activity of this type of compounds, stereoselective strategies

for the functionalization of the steroid backbone, mainly in positions 4 to 6 are now challenging goals.^[5]

An important methodology is the epoxidation of unsaturated Δ^4 - and Δ^5 -steroids, which has been performed either directly or in a catalytic way, leading to different stereoselected products. Furthermore, the careful choice of an agent for the oxirane-ring opening allows the introduction of several functionalities into specific positions.^[6–10]

Direct epoxidation of both Δ^4 - and Δ^5 -steroids with organic peroxy acids affords mainly α -epoxides,^[11] except in the particular case when a β -hydroxy or an analogous group is in the allylic position; in such cases *syn* stereodirecting effects have been observed, leading mainly to the β -epoxides.^[12,13] The catalytic epoxidation of Δ^4 - and Δ^5 -steroids leads mainly to β -epoxidation.^[14–16]

Metalloporphyrins have been found to be efficient catalysts for the epoxidation of alkenes with several oxidants.^[17] Among the oxidants used, hydrogen peroxide allows a clean chemistry, since water is the only byproduct obtained;^[18–20] such environmentally friendly procedures are now imperative in chemical synthesis. The application of Ru porphyrins in the oxidation of Δ^5 -steroids of the cholestane series by O_2 has been described; β -epoxides were obtained and the conversion and stereoselectivity of the reaction were found to be strongly dependent on the type of substituent in the 3-position.^[21–24] During our studies^[25] the oxidation of 5-

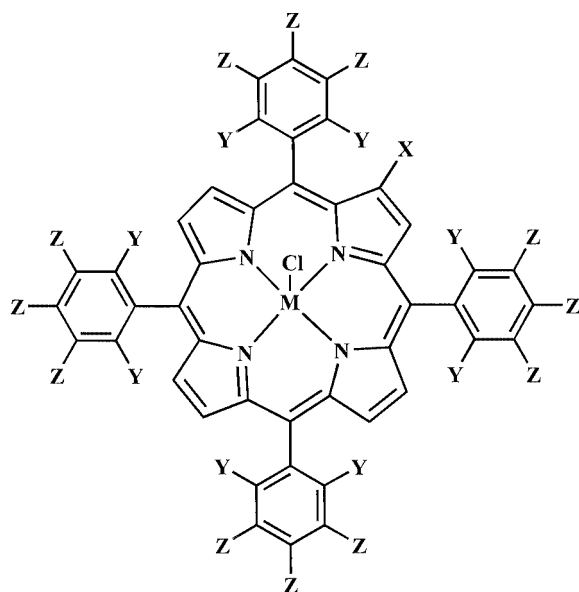
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cholestene derivatives by PhIO with a supported electron-donating Mn^{III} porphyrin was also found to afford β -epoxides.^[26]

We report herein the oxidation of 17 β -acetoxy-4-androstene (**1**), 4-cholestene (**2**) and 3 β -acetoxy-5-cholestene (**3**) with H_2O_2 , in the presence of several Mn^{III} and Fe^{III} porphyrins (Figure 1). The results are discussed on the basis of a comparative study using *m*-CPBA (*m*-chloroperbenzoic acid) as a standard oxidant.



- I: $\text{Mn}(\text{TDCPP})\text{Cl}$ $\text{M} = \text{Mn}; \text{X} = \text{H}; \text{Y} = \text{Cl}; \text{Z} = \text{H}$
 II: $\text{Mn}(\beta\text{NO}_2\text{TDCPP})\text{Cl}$ $\text{M} = \text{Mn}; \text{X} = \text{NO}_2; \text{Y} = \text{Cl}; \text{Z} = \text{H}$
 III: $\text{Mn}(\text{TPFPP})\text{Cl}$ $\text{M} = \text{Mn}; \text{X} = \text{H}; \text{Y} = \text{Z} = \text{F}$
 IV: $\text{Fe}(\text{TPFPP})\text{Cl}$ $\text{M} = \text{Fe}; \text{X} = \text{H}; \text{Y} = \text{Z} = \text{F}$

Figure 1. Metalloporphyrin complexes used in these studies

Results and Discussion

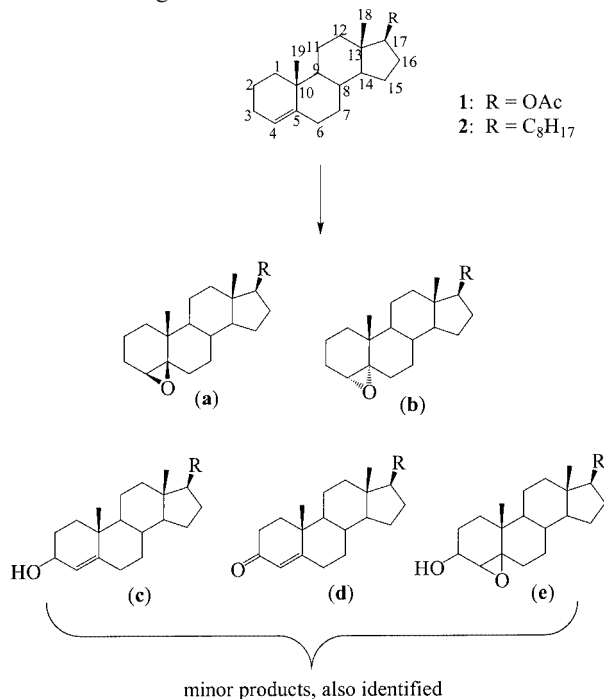
Oxidation of Substrates

The oxidation reactions were carried out at room temperature, with progressive addition of H_2O_2 , in the presence of the selected metalloporphyrin (Figure 1). The reactions were followed by GC every 30 min and the addition of H_2O_2 was stopped when the proportion of the compounds remained constant after two successive GC analysis. The final results were based on the NMR spectra of the total reaction mixtures (see Exp. Sect.).

Oxidation reactions carried out with the manganese(III) porphyrins were performed in a $\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$ mixture, with ammonium acetate as the co-catalyst, which is essential for the heterolytic cleavage of H_2O_2 .^[27] When the iron(III) catalyst was used the reactions were carried out in $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ and no co-catalyst was added.^[28,29]

Oxidation of 17 β -Acetoxy-4-androstene (**1**)

Oxidation of **1** was performed with H_2O_2 in the presence of the catalysts **I–IV** and also with *m*-CPBA alone for comparative purposes (Scheme 1 and Table 1). The products were isolated from the reaction mixtures by preparative TLC on silica gel.



Scheme 1

The structure and stereochemistry of epoxides 17 β -acetoxy-4,5 β -epoxyandrostane (**1a**) and 17 β -acetoxy-4,5 α -epoxyandrostane (**1b**) were identified by GC-MS, NMR spectroscopy and X-ray crystallography. It is worth pointing out that the characterization of this type of compounds has been poorly described in the literature.^[15,16]

The full assignment of the ^1H and ^{13}C NMR signals (Table 2) was achieved by two-dimensional techniques, namely gCOSY ($^1\text{H}/^1\text{H}$), gHSQC ($^1\text{H}/^{13}\text{C}$) and gHMBC ($^1\text{H}/^{13}\text{C}$ -long range) experiments, as well as by DEPT techniques. The stereochemistry of the α - and β -epoxides was unambiguously identified by X-ray crystallography (Figure 2 and 3).

Compound **1a** crystallizes in the chiral space group $P2_1$ with two symmetry-related molecules (formula unit: $\text{C}_{21}\text{H}_{32}\text{O}_3$) in the unit cell. In the chiral molecule all the rings are *trans*-fused, with the exception of A/B, which have a *cis*-junction [torsion angles $\text{C}(1)–\text{C}(10)–\text{C}(5)–\text{C}(6)$: $174.90(19)^\circ$; $\text{C}(19)–\text{C}(10)–\text{C}(5)–\text{C}(4)$: $138.0(2)^\circ$]. Ring A has a distorted sofa conformation with the substituent O(1) atom bonded to C(4) and C(5) in a nearly bisecting position. The O–C bond lengths are 1.444(4) and 1.443(3) Å for C(4) and C(5), respectively, and the angles around C(5) are $115.10(19)^\circ$ [$\text{O}(1)–\text{C}(5)–\text{C}(10)$] and $118.1(2)^\circ$ [$\text{C}(4)–\text{C}(5)–\text{C}(6)$]. Rings B and C have normal, slightly flattened chair conformations, and in the five-membered ring O(2) bonds to C(17) in an equatorial position.

Table 1. Oxidation of 17 β -acetoxy-4-androstene (**1**) with H₂O₂ in the presence of different porphyrin catalysts

Entry	Catalyst	Time (h)	Conversion (%)	1a (β)	1b (α)	Yield (%) 1c	1d	1e	ES ^[a] $\beta/(\alpha+\beta)$
1	[Mn(TDCPP)Cl] (I) ^[b]	1	88	49	18	3	5	8	73
2	[Mn(TDCPP)Cl] (I) ^[b]	2.5	98	43	15	0	6	32	74
3	[Mn(β NO ₂ TDCPP)Cl] (II) ^[b]	1	74	25	8	13	10	8	76
4	[Mn(β NO ₂ TDCPP)Cl] (II) ^[b]	2	96	31	10	3	18	25	76
5	[Mn(TPFPP)Cl] (III) ^[b]	2	79	34	29	3	4	8	54
6	[Fe(TPFPP)Cl] (IV) ^[c]	0.5	81	24	47	0	0	0	34
7	[Fe(TPFPP)Cl] (IV) ^[c]	1	100	33	57	0	0	0	37
8	<i>m</i> -CPBA ^[d]	1	99	33	51	5	0	0	39
9	no catalyst	4	0	—	—	—	—	—	—

^[a] Epoxidation selectivity, measured by the proportion of the β -epoxide (**1a**) with respect to the total amount of epoxides **1a** and **1b**. ^[b] Reaction conditions: substrate (0.03 mmol), catalyst (0.2 μ mol) and ammonium acetate (0.08 mmol) were dissolved in 2 mL of CH₃CN/CH₂Cl₂ (1:1) and stirred at room temperature. Aqueous H₂O₂ (30% w/w) diluted with CH₃CN (1:20) was added to the reaction mixture in 30 μ L aliquots every 15 min. ^[c] Reaction conditions: as in footnote b but without ammonium acetate and the reaction medium was CH₂Cl₂/CH₃OH (1:3). ^[d] Reaction carried out without catalyst and with *m*-CPBA as oxidant instead of H₂O₂.

Table 2. ¹H and ¹³C NMR spectroscopic data (at 500.13 and 125.77 MHz, respectively) of epoxides **1a** and **1b**^[a]

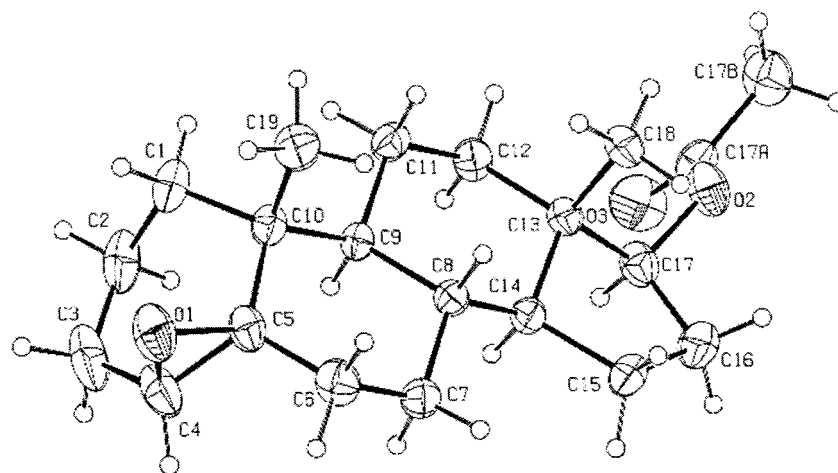
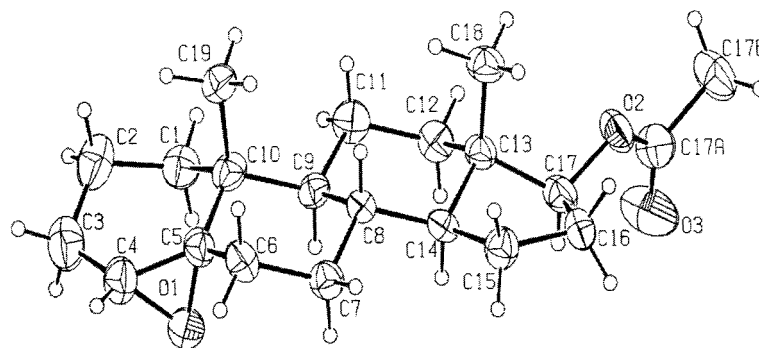
C	17 β -Acetoxy-4,5 β -epoxiandrostan-3-ol (1a)		17 β -Acetoxy-4,5 α -epoxiandrostan-3-ol (1b)	
	¹³ C (δ)	¹ H (δ)	¹³ C (δ)	¹ H (δ)
C-1	29.6	1.25–1.30 (m, 2 H)	31.2	1.18–1.24 (m, 1 H), 1.29–1.33 (m, 1 H)
C-2	15.3	1.23–1.30 (m, 2 H)	15.9	1.35–1.42 (m, 2 H)
C-3	23.6	1.82–1.87 (m, 1 H), 1.89–1.95 (m, 1 H)	22.2	1.83–1.88 (m, 2 H)
C-4	61.4	2.90 (d, <i>J</i> = 4.6 Hz, 1 H)	60.8	2.93 (d, <i>J</i> = 3.9 Hz, 1 H)
C-5	65.5	—	65.9	—
C-6	31.3	0.99–1.07 (m, 1 H), 2.07–2.15 (m, 1 H)	30.1	0.94–0.98 (m, 1 H), 2.00–2.06 (m, 1 H)
C-7	30.0	0.99–1.05 (m, 1 H), 1.75–1.79 (m, 1 H)	27.9	1.26–1.28 (m, 1 H), 1.60–1.62 (m, 1 H)
C-8	34.9	1.48–1.53 (m, 1 H)	35.3	1.56–1.60 (m, 1 H)
C-9	46.4	1.20–1.24 (m, 1 H)	50.1 ^[b]	1.13–1.18 (m, 1 H)
C-10	36.4	—	35.6	—
C-11	20.7	1.32–1.35 (m, 1 H), 1.46–1.50 (m, 1 H)	20.2	1.23–1.27 (m, 1 H), 1.48–1.50 (m, 1 H)
C-12	36.8	1.13–1.20 (m, 1 H), 1.73–1.76 (m, 1 H)	36.8	1.18–1.22 (m, 1 H), 1.72–1.75 (m, 1 H)
C-13	42.6	—	42.6	—
C-14	50.5	1.08–1.13 (m, 1 H)	50.3 ^[b]	1.13–1.18 (m, 1 H)
C-15	23.6	1.31–1.36 (m, 1 H), 1.63–1.69 (m, 1 H)	23.5	1.29–1.34 (m, 1 H), 1.62–1.69 (m, 1 H)
C-16	27.5	1.49–1.53 (m, 1 H), 2.16–2.20 (m, 1 H)	27.5	1.47–1.51 (m, 1 H), 2.15–2.20 (m, 1 H)
C-17	82.7	4.60 (dd, <i>J</i> = 0.9 and 17.0 Hz, 1 H)	82.8	4.60 (dd, <i>J</i> = 1.3 and 17.0 Hz, 1 H)
C-18	12.0	0.81 (s, 3 H)	12.1	0.81 (s, 3 H)
C-19	19.2	1.01 (s, 3 H)	17.4	1.07 (s, 3 H)
CO ₂ CH ₃	171.2	—	171.2	—
CO ₂ CH ₃	21.2	2.05 (s, 3 H)	21.2	2.04 (s, 3 H)

^[a] Chemical shifts are expressed in ppm and the coupling constants (*J*) in Hz. ^[b] The assignments of these two carbons could be interchangeable.

Compound **1b** crystallizes in the chiral space group *P*2₁2₁2₁ with four symmetry-related molecules (formula unit: C₂₁H₃₂O₃) in the unit cell. In the chiral molecule all the rings are *trans*-fused. The torsion angles around the A/B junction are 178.3(10)° [C(1)–C(10)–C(5)–C(6)] and 94.7(12)° [C(19)–C(10)–C(5)–C(4)]. Ring A has a distorted sofa conformation with the substituent O(1) atom bonded to C(4) and C(5) in a nearly bisectonal position. The O–C bond lengths are 1.473(13) and 1.442(13) Å for C(4) and C(5), respectively, and the angles around C(5) are 114.3(9)° [O(1)–C(5)–C(10)] and 120.4(11)° [C(4)–C(5)–C(6)]. Rings B and C have normal slightly flattened chair conformations, and in the five-membered ring O(2) the bonds to C(17) are in the equatorial positions.

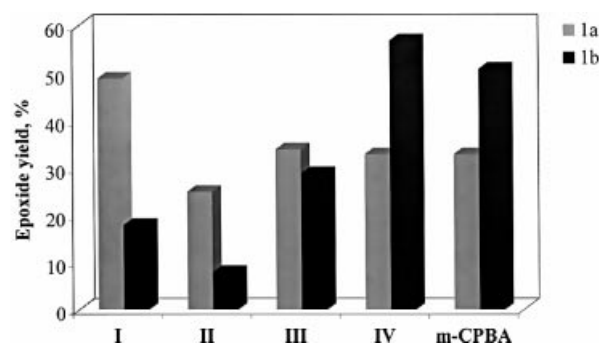
There are another three isolated products, identified by GC-MS and ¹H and ¹³C NMR spectroscopy, as 17 β -acetoxy-4-androsten-3-ol (**1c**), 17 β -acetoxy-4-androsten-3-one (**1d**) and 17 β -acetoxy-4,5-epoxyandrostan-3-ol (**1e**).

The catalytic results are shown in Table 1 and these data clearly indicate that the oxidation efficiency is dependent on the catalyst used and on the reaction time. Maximum conversions of 98, 96, 79 and 100% were obtained with [Mn(TDCPP)Cl], [Mn(β NO₂TDCPP)Cl], [Mn(TPFPP)Cl] and [Fe(TPFPP)Cl], respectively. With manganese porphyrins, the fastest reaction was obtained with [Mn(TDCPP)Cl], which gave rise to 88% conversion after one hour; however, in an equal time period, [Fe(TPFPP)Cl] gave complete conversion of substrate.

Figure 2. ORTEP^[44] diagram of compound **1a** drawn at the 30% probability levelFigure 3. ORTEP^[44] diagram of compound **1b** drawn at the 30% probability level

The maximum yield of the β -epoxide (**1a**) formation was achieved with [Mn(TDCPP)Cl] after 1 h of reaction (49%, Table 1, entry 1). With this catalyst we also observed an increase in the yield of compound **1e** from 8 to 32% when the reaction time was increased from 1 h to 2.5 h (entry 2). Despite the high β -stereoselectivity observed in the reactions catalyzed by [Mn(β NO₂TDCPP)Cl], lower yields of epoxides were found since this catalyst seems to promote allylic oxidation, producing the highest yields of alcohol **1c** (13%) and ketone **1d** (18%) after 1 h and 2 h of reaction, respectively (entries 3 and 4). Also in this case, after 2 h of reaction, compound **1e** was obtained in 25% yield. Moderate yields for the epoxides were observed with [Mn(TPFPP)Cl], but nearly equal amounts of β - and α -epoxides were obtained (entry 5).

A change in stereoselectivity was observed in the presence of [Fe(TPFPP)Cl], since this reaction took place with higher selectivity for the α -epoxide **1b**. With this catalyst, and after 1 h of reaction, total conversion and 57% yield of **1b** were observed (Table 1, entry 7). This catalyst shows an oxidation pattern similar to that due to *m*-CPBA, where the α -epoxide was obtained in 51% yield (entry 8). The epoxidation stereoselectivity distribution can be more clearly observed in Figure 4.

Figure 4. Oxidation of 17 β -acetoxy-4-androstene (**1**) with H₂O₂ in the presence of catalysts **I–IV**, after one hour reaction time; the results are compared with those from stoichiometric *m*-CPBA oxidation

Oxidation of 4-Cholestene (**2**)

The oxidation of Δ^4 -steroid **2** was also performed with H₂O₂ in the presence of the catalysts **I–IV**, as well as with *m*-CPBA for comparative purposes (Table 3).

Table 3. Oxidation of 4-cholestene **2** with H₂O₂ in the presence of different porphyrin catalysts

Entry	Catalyst	Time (h)	Conversion (%)	2a (β)	2b (α)	Yield (%)			ES ^[a] β/(β+α)
1	[Mn(TDCPP)Cl] (I) ^[b]	1	84	42	17	6	5	9	71
2	[Mn(TDCPP)Cl] (I) ^[b]	2.5	96	41	16	2	8	25	72
3	[Mn(βNO ₂ TDCPP)Cl] (II) ^[b]	1	72	27	11	11	9	8	71
4	[Mn(βNO ₂ TDCPP)Cl] (II) ^[b]	2	95	27	11	5	21	24	71
5	[Mn(TPFPP)Cl] (III) ^[b]	2	79	34	29	4	3	6	54
6	[Fe(TPFPP)Cl] (IV) ^[c]	0.5	80	25	44	0	0	0	36
7	[Fe(TPFPP)Cl] (IV) ^[c]	1	98	33	54	0	0	0	38
8	<i>m</i> -CPBA ^[d]	1	94	36	50	4	1	0	42
9	no catalyst	4	0	—	—	—	—	—	—

^[a] Epoxidation selectivity, measured by the proportion of the β-epoxide (**2a**) with respect to the total amount of epoxides **2a** and **2b**. ^[b] Reaction conditions: substrate (0.03 mmol), catalyst (0.2 μmol) and ammonium acetate (0.08 mmol) were dissolved in 2 mL of CH₃CN/CH₂Cl₂ (1:1) and stirred at room temperature. Aqueous H₂O₂ (30% w/w) diluted with CH₃CN (1:20) was added to the reaction mixture in 30 μL aliquots every 15 min. ^[c] Reaction conditions: as in footnote b but without ammonium acetate and the reaction medium was CH₂Cl₂/CH₃OH (1:3). ^[d] Reaction carried out without catalyst and with *m*-CPBA as oxidant instead of H₂O₂.

The product mixtures resulting from substrate **2** oxidation reactions were fractionated by preparative TLC on silica gel; the isolated products (Scheme 1) were 4,5β-epoxycholestane (**2a**), 4,5α-epoxycholestane (**2b**), 4-cholesten-3-ol (**2c**), 4-cholesten-3-one (**2d**) and 4,5-epoxycholestan-3-ol (**2e**), which were fully characterized by GC-MS and ¹H and ¹³C NMR spectroscopy.

The assignment of the resonances in the ¹H and ¹³C NMR spectra of compounds **2a** and **2b** was achieved using

two-dimensional techniques, namely gCOSY (¹H/¹H), gHSQC (¹H/¹³C) and gHMBC (¹H/¹³C-long range) experiments, as well as by DEPT techniques. The results are collected in Table 4.

The stereochemistry of the epoxides **2a** and **2b** was established by comparison of their NMR spectra with those described for the 4,5-epoxides of the androstane series (**1a** and **1b**). Previous literature data reported the partial characterization of these compounds,^[7,10,11] but from these data

Table 4. ¹³C and ¹H NMR spectroscopic data (at 75.47 and 300.13 MHz, respectively) of epoxides **2a** and **2b**^[a]

C	4,5β-Epoxycholestane (2a)		4,5α-Epoxycholestane (2b)	
	¹³ C (δ)	¹ H (δ)	¹³ C (δ)	¹ H (δ)
C-1	29.6	1.25–1.32 (m, 2 H)	31.1	1.19–1.24 (m, 2 H)
C-2	15.3	1.20–1.33 (m, 2 H)	15.9	1.35–1.41 (m, 2 H)
C-3	23.7	1.82–1.94 (m, 2 H)	22.3	1.85–1.88 (m, 2 H)
C-4	61.5	2.89 (d, <i>J</i> = 4.3 Hz, 1 H)	60.7	2.92 (d, <i>J</i> = 3.0 Hz, 1 H)
C-5	65.7	—	66.1	—
C-6	31.5	0.97–1.02 (m, 1 H), 2.09 (dt, <i>J</i> = 4.1 and 14.2 Hz, 1 H)	30.2	0.94–0.99 (m, 1 H), 2.00–2.06 (m, 1 H)
C-7	30.6	0.96–1.05 (m, 1 H), 1.71–1.80 (m, 1 H)	28.4	1.25–1.30 (m, 1 H), 1.60–1.62 (m, 1 H)
C-8	35.1	1.41–1.47 (m, 1 H)	35.5	1.58–1.60 (m, 1 H)
C-9	46.4	1.18–1.25 (m, 1 H)	50.2	1.13–1.19 (m, 1 H)
C-10	36.2	—	35.5	—
C-11	23.8	1.11–1.14 (m, 1 H), 1.32–1.40 (m, 1 H)	23.8	1.11–1.16 (m, 1 H), 1.30–1.38 (m, 1 H)
C-12	39.8	1.99 (dt, <i>J</i> = 3.2 and 12.5 Hz, 2 H)	39.8	1.98–2.01 (m, 2 H)
C-13	42.6	—	42.6	—
C-14	56.2	1.11–1.14 (m, 1 H)	55.7	1.11–1.14 (m, 1 H)
C-15 ^[b]	21.3	1.32–1.47 (m, 2 H)	20.7	1.30–1.41 (m, 2 H)
C-16 ^[b]	24.3	1.60–1.64 (m, 2 H)	24.1	1.58–1.62 (m, 2 H)
C-17	56.2	0.97–1.09 (m, 1 H)	56.1	1.00–1.09 (m, 1 H)
C-18	12.0	0.68 (s, 3 H)	12.0	0.68 (s, 3 H)
C-19	19.2	1.00 (s, 3 H)	17.4	1.05 (s, 3 H)
C-20	35.8	0.96–1.05 (m, 1 H)	36.1	0.96–1.05 (m, 1 H)
C-21	18.6	0.91 (d, <i>J</i> = 6.5 Hz, 3 H)	18.6	0.90 (d, <i>J</i> = 6.5 Hz, 3 H)
C-22	36.2	1.32–1.40 (m, 2 H)	36.2	1.32–1.40 (m, 2 H)
C-23	28.2	1.81–1.88 (m, 2 H)	28.2	1.85–1.87 (m, 2 H)
C-24	39.5	1.10–1.18 (m, 2 H)	39.5	1.10–1.16 (m, 2 H)
C-25	28.0	1.47–1.54 (m, 1 H)	28.0	1.47–1.54 (m, 1 H)
C-26 ^[c]	22.6	0.863 (d, <i>J</i> = 6.6 Hz, 3 H)	22.6 ^[d]	0.87 (d, <i>J</i> = 6.6 Hz, 3 H)
C-27 ^[c]	22.8	0.867 (d, <i>J</i> = 6.6 Hz, 3 H)	22.8 ^[d]	0.85 (d, <i>J</i> = 6.6 Hz, 3 H)

^[a] Chemical shifts are expressed in ppm and the coupling constants (*J*) in Hz. ^[b] The assignments of these two carbons could be interchangeable. ^[c] The assignments of these two carbons could be interchangeable. ^[d] The assignments of these two carbons could be interchangeable.

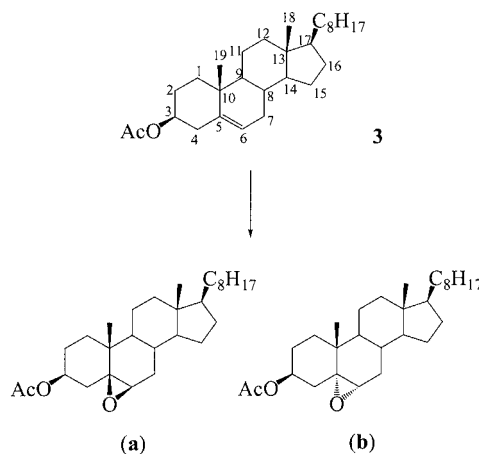
we were unable to distinguish between the two epoxides due to incoherent NMR results.^[10,11] With the techniques now available (NMR spectroscopy and X-ray crystallography) we concluded that several previous NMR assignments are not correct. The chemical shift of H-4 in 4,5 β -epoxycholestane (**2a**) was unequivocally assigned at δ = 2.89 ppm instead of δ = 2.98 ppm.^[11] The assignment of the carbon resonances of 4,5 α -epoxycholestane (**2b**)^[10] was corrected for C-2 (δ = 15.9 instead of 22.5 ppm), C-3 (δ = 22.3 instead of 28.5 ppm) and C-19 (δ = 17.4 instead of 16.0 ppm). The assignment of the carbon resonances of 4,5 β -epoxycholestane (**2a**)^[10] was corrected for C-2 (δ = 15.3 instead of 17.4 ppm) and for C-10 (δ = 36.2 instead of 35.2 ppm). The unequivocal assignment of all resonances of compounds **2a** and **2b** is given in Table 4.

The behavior towards oxidation of 4-cholestene by H₂O₂, catalyzed by metalloporphyrins, is similar to that observed with steroid **1**. The catalytic results collected in Table 3 show that the oxidation efficiency depends on the catalyst used and on the reaction time. Maximum conversions of 96, 95, 79 and 98% were obtained with [Mn(TDCPP)Cl], [Mn(β NO₂TDCPP)Cl], [Mn(TPFPP)Cl] and [Fe(TPFPP)Cl], respectively. The fastest reaction occurred in the presence of catalyst **IV**, followed by the reaction catalyzed by catalyst **I**, which reached 98 and 84% conversion, respectively, after one hour of reaction.

Despite these differences, epoxidation is the main pathway observed in all reactions. For synthetic purposes [Mn(TDCPP)Cl] is the best catalyst to obtain the 4,5 β -epoxide (**2a**): a 42% yield of this compound was achieved after 1 h of reaction time (entry 1). With the same catalyst a longer reaction time (2.5 h) afforded a 25% yield of the epoxy alcohol (**2e**). 4,5 α -Epoxycholestane (**2b**) was obtained as the major product with [Fe(TPFPP)Cl] (entry 7); these results are similar to the ones obtained with *m*-CPBA (entry 8), although the iron porphyrin complex is slightly more selective for the epoxidation reaction. Although high conversions are achieved with [Mn(β NO₂TDCPP)Cl], this is not a catalyst of choice for these epoxidations due to the high yields of allylic alcohol and ketone obtained. Catalyst **III** presents the lowest stereoselectivity observed in the epoxidation of 4-cholestene.

Oxidation of 3 β -Acetoxy-5-cholestene (**3**)

The two epoxides resulting from the oxidation of substrate **3** (Scheme 2) were isolated from the reaction mixture by column chromatography and identified by comparing their ¹H NMR spectra with literature data.^[23] The signal of the 6 β proton of the 5,6 α -epoxycholestane appears as a doublet at δ = 2.9 ppm (J = 4 Hz), and is clearly distinguishable from the doublet at δ = 3.1 ppm (J = 2 Hz) for the 6 α proton of the 5,6 β -epoxide. The mass spectra of these compounds show that a loss of acetic acid occurs in the injector, as the highest *m/z* values observed correspond to [M⁺ – 60] ions.



Scheme 2

Oxidation of steroid **3** in the presence of catalysts **I–IV** proceeds with 100% chemoselectivity for epoxidation (Table 5). High conversions are obtained with [Mn(TDCPP)Cl] and [Mn(β NO₂TDCPP)Cl] (79 and 80%, respectively) and these reactions lead to high β -stereoselectivity. [Mn(TDCPP)Cl] is the best catalyst for β -epoxidation, affording compound **3a** in 71% yield and with 90% β -selectivity.

The reactions with [Fe(TPFPP)Cl] and H₂O₂ or *m*-CPBA without catalyst gave 100% conversion, but lower stereoselectivities. In the reaction catalyzed by the iron porphyrin

Table 5. Oxidation of 3 β -acetoxy-5-cholestene (**3**) with H₂O₂ in the presence of different porphyrin catalysts

Entry	Catalyst	Time (h)	Conversion (%)	Yield (%) 3a (β)	3b (α)	ES ^[a] $\beta/(\beta+\alpha)$
1	[Mn(TDCPP)Cl] (I) ^[b]	3	79	71	8	90
2	[Mn(β NO ₂ TDCPP)Cl] (II) ^[b]	4	80	66	14	82
3	[Mn(TPFPP)Cl] (III) ^[b]	3	43	31	12	72
4	[Fe(TPFPP)Cl] (IV) ^[c]	2	100	66	34	66
5	<i>m</i> -CPBA ^[d]	2	100	43	57	43
6	no catalyst	5	0	—	—	—

^[a] Epoxidation selectivity, measured by the proportion of the β -epoxide (**3a**) with respect to the total amount of epoxides **3a** and **3b**. ^[b] Reaction conditions: substrate (0.03 mmol), catalyst (0.2 μ mol) and ammonium acetate (0.08 mmol) were dissolved in 2 mL of CH₃CN/CH₂Cl₂ (1:1) and stirred at room temperature. Aqueous H₂O₂ (30% w/w) diluted with CH₃CN (1:20) was added to the reaction mixture in 30 μ L aliquots every 15 min. ^[c] Reaction conditions as in footnote b but without ammonium acetate and the reaction medium was CH₂Cl₂/CH₃OH (1:3). ^[d] Reaction carried out without catalyst and with *m*-CPBA as oxidant instead of H₂O₂.

IV a β -selectivity of 66% was achieved. The stoichiometric *m*-CPBA oxidation afforded a selectivity of 43% for the β -epoxide **3b** and 57% for the α -isomer (Figure 5).

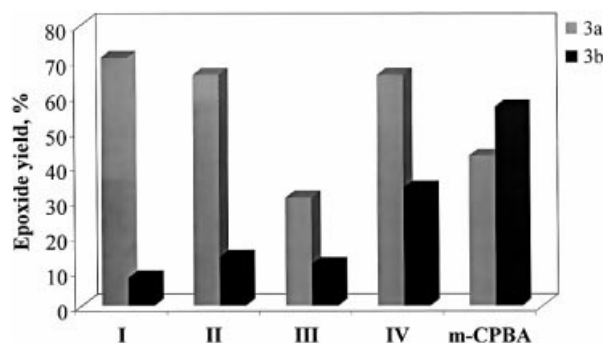


Figure 5. Oxidation of 3- β -acetoxy-5-cholestene (**3**) with H_2O_2 in the presence of catalysts **I**–**IV**; the results are compared with those from stoichiometric *m*-CPBA oxidation

Mechanistic Considerations

The stereochemical differences observed in the epoxidation of Δ^4 -steroids by H_2O_2 in the presence of different metalloporphyrins point to the possibility that different mechanisms and intermediate species could be present. It is now generally accepted that oxidation reactions catalyzed by metalloporphyrins with oxidants such as PhIO, *m*-CPBA, KHSO_5 or NaOCl proceed through a short cycle of the cytochrome P450-type mechanism, with the formation of high-valent oxo species, which seem to be the final oxidant species (**B** in Scheme 3). Many theoretical studies and the detection of the elusive metalloporphyrin oxo species by stopped-flow visible spectrometry have corroborated this hypothesis.^[30,31] However, the mechanism of oxidation reactions with H_2O_2 or alkyl hydroperoxides in the presence of metalloporphyrins has been more controversial because, in the presence of these oxidants, the intermediate species **A** (Scheme 3) can undergo heterolytic or homolytic cleavage,

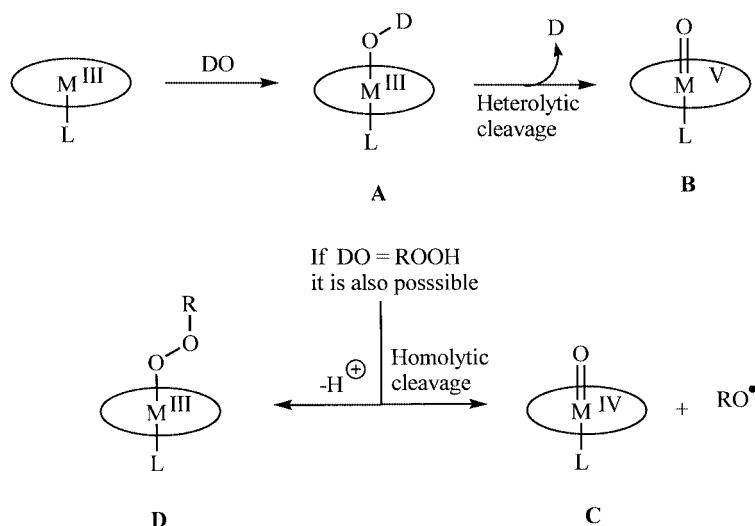
or it can even act as a metalloperoxy complex as the final oxidant (species **D**).^[32]

Although in some cases indirect evidence suggests that homolytic cleavage can occur with alkyl hydroperoxides, to generate RO^\bullet radicals at low temperature, which can initiate a radical chain oxidation mechanism,^[33,34] many reported results also show that with H_2O_2 in the presence of Mn^{III} porphyrins like $[\text{Mn}(\text{TDCPP})\text{Cl}]$ and a buffering co-catalyst, a manganese oxo complex is the final active species, and therefore heterolytic cleavage takes place. Evidence for that is: (a) the type of reactivity (conversion and selectivity in several substrates oxidation) demonstrated by the system ($\text{MnTDCPP}/\text{H}_2\text{O}_2/\text{imidazole}$) is identical to that observed with the system ($\text{MnTDCPP}/\text{PhIO}/\text{imidazole}$) and this points out to the involvement of the same final active species;^[19] (b) the change from almost no reactivity to high reactivity when the $[\text{Mn}(\text{TDCPP})\text{Cl}]/\text{H}_2\text{O}_2$ system is used in the absence or in the presence of a buffering co-catalyst which can contribute to the heterolytic cleavage by basic and acid catalysis;^[20,27] or (c) the preferential epoxidation of unreactive aromatic hydrocarbons like naphthalene by the $[\text{Mn}(\text{TDCPP})\text{Cl}]/\text{H}_2\text{O}_2/\text{NH}_4\text{OAc}$ system, which is very unlikely in a radical-type mechanism.^[35]

In the conditions used here for Mn^{III} porphyrin catalysis, the heterolytic cleavage of hydrogen peroxide probably occurs and a manganese oxo species is the final oxidant; this species is capable of both double-bond epoxidation and hydroxylation of activated allylic C–H bonds.

In the case of $[\text{Fe}(\text{TPFPP})\text{Cl}]$, which shows identical reactivity to *m*-CPBA, we consider it likely that under the reaction conditions used (protic solvent/no buffering co-catalyst) the final oxidation species is a metalloperoxy species (**D** in Scheme 3; $\text{R} = \text{H}$). This kind of species has been described to be inert towards alkanes, but is probably reactive enough to epoxidise alkenes.^[36]

Further evidence for these two different mechanisms was obtained in the visible spectra of the reaction mixtures: Mn^{III} porphyrins, with Soret bands near 480 nm, are con-



Scheme 3

verted, during the course of the reaction, into another species with a brief lifetime and with Soret bands close to 430 nm; the Soret band of [Fe(TPFPP)Cl] is maintained close to 410 nm during the whole reaction.

The stereochemical hindrance of the substrates and the different intervening catalytic species referred to above can also justify the approach of the metalloporphyrin complex to the substrate from either the α or the β side. In Δ^4 -steroids, the most stable folded structure along the A and B ring-fusion bond has previously been invoked to explain the formation of β products.^[37] Therefore, the porphyrin oxo species should approach the double bond preferably from the *cis* side, producing preferentially the β -epoxides, whereas metalloperoxyporphyrin species having a peripheral atom bridge, analogous to *m*-CPBA, can approach the double bond from the *trans* side, giving rise to α -epoxides as the main products.

We consider that [Mn(TDCPP)Cl] activates the hydrogen peroxide mainly through the formation of a Mn^{V} oxo species, leading mainly to β -epoxidation. The differences observed for the performances of [Mn(TDCPP)Cl] and [Mn(TPFPP)Cl] could be explained by a more difficult formation of this oxo species in the [Mn(TPFPP)Cl] case, due to its higher redox potential. In this case, both oxo and peroxy species could be active. The differences between the two catalysts could also be explained by the smaller substituents on [Mn(TPFPP)Cl], which lead to a lower stereoselectivity. In the oxidation of the Δ^5 -steroid, the approach of the catalyst from the *trans* side to afford the α -epoxide is even more hindered, and this can justify the lower reaction conversions and higher β -selectivities reported with all the metalloporphyrins. On the other hand, *m*-CPBA is a small molecule with a peroxy atom bridge, and it can approach the double bond from both sides.

Conclusions

The epoxidation of Δ^4 - and Δ^5 -steroids of the androstane and cholestane series proceeds efficiently in the presence of metalloporphyrin catalysts under mild conditions and using an ecologically safe oxidant such as hydrogen peroxide.

The system H_2O_2 /[Mn(TDCPP)Cl] or [Mn(βNO_2 -TDCPP)Cl]/ NH_4AcO in $\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$ performs the epoxidation of Δ^4 - and Δ^5 -steroids in a β -stereoselective way. In the reactions of Δ^4 -steroids **1** and **2** epoxidation was always the main pathway, but allylic alcohols and ketones were also observed in minor amounts, especially with [Mn(βNO_2 -TDCPP)Cl]. Catalyst **I** afforded the highest yields of β -epoxides. For conversions above 90%, significant and increasing quantities of 4,5-epoxy-3-hydroxysteroids were also observed with catalysts **I** and **II**. Catalyst **III** was the least stereoselective. In the reaction of the Δ^5 -steroid **3**, 100% chemoselectivity for the epoxidation and higher β -selectivities were observed.

High conversions were observed with the system H_2O_2 /[Fe(TPFPP)Cl] in $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$. In oxidation of Δ^4 -steroids the stereochemistry was inverted, and α -epoxides were

the major compounds produced, similar to the stoichiometric oxidation by *m*-CPBA. In the oxidation of Δ^5 -steroids, an increase in the α -face attack was observed when catalyst **IV** was used.

These results have been rationalized in terms of the active metalloporphyrin catalytic species together with the stereochemical hindrance of the substrate, and we have proposed the involvement of an oxo species for the β approach and a peroxy species for the α approach. Considering recent publications on the possible existence of multiple active oxygenating species in porphyrin-catalyzed oxidations,^[43] the present results give further insight into such a hypothesis when hydrogen peroxide is used as the oxidant.

The main products were fully characterized by single-crystal X-ray diffraction and double resonance NMR techniques; the results constitute an important and clarifying study of this type of compounds.

Further studies on the epoxidation reaction mechanism, in order to disclose the effect of the porphyrin structure and the central metal on the catalytically active species, are currently being carried out using different catalysts and substrates. The results will be published in due course.

Experimental Section

General Details: ^1H and ^{13}C NMR spectra were acquired at 22 °C in CDCl_3 solutions at 500.13 MHz or 300.13 MHz and at 125.76 or 75.47 MHz, respectively, using Bruker DRX 500 and 300 spectrometers. The chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference. GC-MS analyses were performed using a Finnigan Trace GC-MS (Thermo Quest CE instruments) using helium as the carrier gas (35 cm/s). GC-FID analyses were performed using a Varian Star 3400 CX gas chromatograph and hydrogen as the carrier gas (55 cm/s). In both cases fused silica Supelco capillary columns SPB-5 (30 m \times 0.25 mm i.d.; 0.25 μm film thickness) were used. Gas chromatographic conditions: temperature 250 °C, during 1 min; temperature rate 30 °C/min; final temperature 290 °C during 12 min; injector temperature 290 °C; detector temperature 300 °C.

Preparative thin-layer chromatography (TLC) was carried out on silica gel plates (Riedel-de Haën silica gel 60 DGF₂₅₄). Hydrogen peroxide (30 wt.-% solution in water) and acetonitrile were purchased from Riedel-de Haën. All other chemicals and solvents were obtained from commercial sources and either used as received or distilled and dried using standard procedures. Light petroleum was the fraction with a boiling point of 40–60 °C.

Preparation of Substrates: The substrates were prepared by published procedures. Substrates **1** and **2** were obtained by reduction of testosterone acetate and 4-cholesten-3-one, respectively.^[5] Substrate **3** was prepared by reaction of 5-cholesten-3 β -ol with Ac_2O in pyridine.^[38] The spectroscopic data obtained for **1**, **2** and **3** were identical to those reported in the literature.

Catalyst Synthesis: The free bases of the metalloporphyrins **I–IV** (Figure 1) were prepared according to described procedures.^[39–41] Metallation of the free bases leading to the formation of complexes **I–IV** was performed with MnCl_2 or FeCl_2 according to conventional methods.^[40,42]

Oxidation Reactions with Mn^{III} Porphyrins: In a typical experiment, the substrate (0.03 mmol) and ammonium acetate (0.08 mmol)

were dissolved in 2 mL of a stock solution of the catalyst [0.1 μmol of catalyst per mL of $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ (1:1)] and stirred at room temperature. Aqueous hydrogen peroxide (30% w/w) diluted with acetonitrile (1:20) was added to the reaction mixture in 30 μL aliquots every 15 min.

Oxidation Reactions with Fe^{III} Porphyrins: In a typical experiment, the substrate (0.03 mmol) was dissolved in 2 mL of a stock solution of the catalyst [0.1 μmol of catalyst per mL of $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (3:1)] and stirred at room temperature. Aqueous hydrogen peroxide (30% w/w) diluted with acetonitrile (1:20) was added to the reaction mixture in 30 μL aliquots every 15 min.

Reaction Control: Aliquots were withdrawn from the reaction mixture and injected directly into the GC injector. The addition of H_2O_2 was stopped when the relative proportion of the compounds remained constant after two successive GC analyses. Reaction conversions and product yields were based on the ^1H NMR spectra of the reaction mixture. This type of approach was possible because the various components of the reaction mixture exhibit distinct signals between $\delta = 2$ and 6 ppm. Only the H-4 signals of the two isomers of 4,5-epoxides (Scheme 1) are not distinguishable in that region of the ^1H NMR spectra; their relative abundance was determined by the intensity of the ^1H NMR singlets of H-19 ($\delta = 1.07$ ppm for the α -epoxide and $\delta = 1.01$ ppm for the β -epoxide).

Isolation and Characterization of Reaction Products: The reaction mixture components were separated by preparative thin-layer chromatography on silica gel. Oxidation products from **1** were eluted with a mixture of CH_2Cl_2 /light petroleum (4:1), whereas the oxidation products from **2** were eluted with a mixture of CH_2Cl_2 /light petroleum (1:1). Crystals of epoxides **1a** and **1b**, suitable for X-ray diffraction, were obtained by recrystallization from a mixture of dichloromethane and methanol. Compounds **3a** and **3b** were isolated by column chromatography on silica gel eluting with a (7:3) mixture of CH_2Cl_2 and light petroleum.

17 β -Acetoxy-4,5 β -epoxyandrostane (1a): Retention time (t_R): 2.64 min. MS (EI): m/z (%) = 332 (15) [M^+], 304 (6). M.p. 156.4–157.6 $^\circ\text{C}$. NMR spectroscopic data are given in Table 2.

17 β -Acetoxy-4,5 α -epoxyandrostane (1b): $t_R = 2.74$ min. MS (EI): m/z (%) = 332 (26) [M^+], 304 (15). M.p. 137.6–139.4 $^\circ\text{C}$. NMR spectroscopic data are given in Table 2.

17 β -Acetoxy-4-androsten-3-ol (1c): $t_R = 2.45$ min (dehydration occurs in the injector). MS (EI): m/z (%) = 314 (25) [$\text{M}^+ - 18$], 299 (5). ^1H NMR (300.13 MHz): $\delta = 0.81$ (s, 3 H, 18-H), 0.99 (s, 3 H, 19-H), 2.04 (s, 3 H, 21-H), 4.04–4.09 (m, 1 H, 3-H), 4.58 (dd, $J = 7.9, 9.0$ Hz, 1 H, 17-H), 5.47 (dd, $J = 1.6, 5.0$ Hz, 1 H, 4-H) ppm.

17 β -Acetoxy-4-androsten-3-one (1d): $t_R = 3.57$ min. MS (EI): m/z (%) = 330 (10) [M^+], 288 (15). ^1H NMR (300.13 MHz): $\delta = 0.84$ (s, 3 H, 18-H), 0.99 (s, 3 H, 19-H), 2.05 (s, 3 H, 21-H), 4.58 (dd, $J = 7.9, 9.0$ Hz, 1 H, 17-H), 5.74 (d, $J = 1.1$ Hz, 1 H, 4-H) ppm.

17 β -Acetoxy-3-hydroxy-4,5-epoxyandrostane (1e): $t_R = 3.45$ min (dehydration occurs in the injector). MS (EI): m/z (%) = 330 (15) [$\text{M}^+ - 18$]. ^1H NMR (300.13 MHz): $\delta = 0.81$ (s, 3 H, 18-H), 1.01 (s, 3 H, 19-H), 2.05 (s, 3 H, 21-H), 2.85 (s, 1 H, 4-H), 3.98 (t, $J = 5.6$ Hz, 1 H, 3-H), 4.60 (t, $J = 8.4$ Hz, 1 H, 17-H) ppm.

4,5 β -Epoxycholestane (2a): $t_R = 4.47$ min. MS (EI): m/z (%) = 386 (100) [M^+], 358 (35). NMR spectroscopic data are given in Table 4.

4,5 α -Epoxycholestane (2b): $t_R = 4.84$ min. MS (EI): m/z (%) = 386 (100) [M^+], 358 (35). NMR spectroscopic data are given in Table 4.

4-Cholesten-3-ol (2c): $t_R = 3.96$ min (dehydration occurs in the injector). MS (EI): m/z (%) = 368 (100) [$\text{M}^+ - 18$], 353 (50). ^1H NMR (300.13 MHz): $\delta = 0.68$ (s, 3 H, 18-H), 0.862 and 0.865 (2 d, $J = 6.6$ Hz, 3 \times 2 H, 26-H and 27-H), 0.90 (d, $J = 6.1$ Hz, 3 H, 21-H), 1.00 (s, 3 H, 19-H), 4.05–4.11 (m, 1 H, 3-H), 5.46 (dd, $J = 1.5, 4.8$ Hz, 1 H, 4-H) ppm. ^{13}C NMR (75.47 MHz): $\delta = 64.3$ (C-3), 120.6 (C-4), 150.5 (C-5) ppm.

4-Cholesten-3-one (2d): $t_R = 6.35$ min. MS (EI): m/z (%) = 384 (40) [M^+], 342 (20). ^1H NMR (300.13 MHz): $\delta = 0.67$ (s, 3 H, 18-H), 0.86 (d, $J = 6.6$ Hz, 6 H, 26-H and 27-H), 0.91 (d, $J = 6.4$ Hz, 3 H, 21-H), 1.05 (s, 3 H, 19-H), 5.72 (s-broad, 1 H, 4-H) ppm. ^{13}C NMR (75.47 MHz): $\delta = 123.7$ (C-4), 171.8 (C-5), 119.7 (C-3) ppm.

3-Hydroxy-4,5-epoxycholestane (2e): $t_R = 6.30$ min (dehydration occurs in the injector). MS (EI): m/z (%) = 384 (10) [$\text{M}^+ - 18$]. ^1H NMR (500.13 MHz): $\delta = 0.68$ (s, 3 H, 18-H), 0.868 and 0.872 (2 d, $J = 6.6$ Hz, 2 \times 3 H, 26-H and 27-H), 0.91 (d, $J = 6.5$ Hz, 3 H, 21-H), 1.00 (s, 3 H, 19-H), 1.03–1.09 (m, 1 H, 6-H), 1.05–1.07 (m, 1 H, 22-H), 1.05–1.12 (2m, 2 \times 1 H, 14-H and 17-H), 1.08–1.18 (2m, 1H + 2 H, 12-H and 24-H), 1.14–1.18 (m, 1 H, 1-H), 1.28–1.32 (2m, 2 \times 1 H, 2-H and 9-H), 1.34–1.40 (2m, 2 \times 1 H, 20-H and 22-H), 1.36–1.40 (m, 1 H, 1-H), 1.41–1.46 (2m, 1H + 2 H, 8-H and 11-H), 1.70–1.73 (m, 1 H, 2-H), 1.74–1.79 (m, 2 H, 7-H), 2.00 (dt, $J = 3.3, 12.8$ Hz, 1 H, 12-H), 2.03–2.13 (m, 1 H, 6-H), 2.85 (s, 1 H, 4-H), 3.97 (t, $J = 8.4$ Hz, 1 H, 3-H) ppm. ^{13}C NMR (125.76 MHz): $\delta = 11.9$ (C-18), 18.6 (C-21), 18.7 (C-19), 21.1 (C-11), 22.5 and 22.8 (C-26,27), 23.8 and 24.2 (C-15,16), 25.7 (C-2), 26.0 (C-1), 28.0 (C-25), 28.2 (C-23), 30.2 (C-7), 30.9 (C-6), 35.0 (C-8), 35.7 (C-20), 36.1 (C-22), 36.3 (C-10), 39.4 (C-24), 39.7 (C-12), 42.5 (C-13), 46.0 (C-9), 56.0 and 56.2 (C-14,17), 65.8 (C-4), 66.8 (C-5,3) ppm.

3- β -Acetoxy-5,6- β -epoxycholestane (3a): $t_R = 7.46$ min (loss of acetic acid occurs in the injector). MS (EI): m/z (%) = 384 (14) [$\text{M}^+ - 60$], 356 (8). ^1H NMR (300.13 MHz): $\delta = 0.64$ (s, 3 H, 18-H), 0.858 and 0.861 (2 d, $J = 6.7$ Hz, 2 \times 3 H, 26-H and 27-H), 0.89 (d, $J = 7.7$ Hz, 3 H, 21-H), 1.00 (s, 3 H, 19-H), 2.03 (s, 3 H, 3-OAc), 3.08 (d, $J = 1.6$ Hz, 1 H, 6-H), 4.71–4.82 (m, 1-H, 3-H) ppm.

3- β -Acetoxy-5,6- α -epoxycholestane (3b): $t_R = 8.20$ min (loss of acetic acid occurs in the injector). MS (EI): m/z (%) = 384 (13) [$\text{M}^+ - 60$], 366 (9). ^1H NMR (300.13 MHz): $\delta = 0.61$ (s, 3 H, 18-H), 0.860 and 0.861 (2 d, $J = 6.7$ Hz, 2 \times 3 H, 26-H and 27-H), 0.89 (d, $J = 7.7$ Hz, 3 H, 21-H), 1.07 (s, 3 H, 19-H), 2.02 (s, 3 H, 3-OAc), 2.90 (d, $J = 4.3$ Hz, 1 H, 6-H), 4.89–5.00 (m, 1-H, 3-H) ppm.

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