Singlet-oxygen ene reaction with 3β-substituted stigmastanes. An alternative pathway for the classical Schenck rearrangement

María A. Ponce, Javier A. Ramirez, Lydia R. Galagovsky, Eduardo G. Gros and Rosa Erra-Balsells *

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, 3º Ciudad Universitaria, 1428, Buenos Aires, Argentina

Received (in Cambridge, UK) 10th January 2000, Accepted 11th August 2000
First published as an Advance Article on the web 9th October 2000

The course of the singlet-oxygen ene reaction with stigmasta-5,22-dienes may be controlled if in the substrate a good leaving group as substituent is present at 3-C. Thus when the 5α-hydroperoxystigmasta-5,22-diene is formed instead of the well known allylic rearrangement to yield the 7α-hydroperoxystigmasta-5,22-diene isomer an intramolecular nucleophilic substitution can then occur yielding 5α-hydroxystigmasta-6,22-dien-3-one. Various stigmasta-5,22-dienes were chosen to elucidate which feature of the stigmastane is necessary to control the course of the reaction. Thus, 3β-F, 3β-Cl, 3β-Br, 3β-I and 3α-Br-stigmasta-5,22-dienes were firstly prepared and fully characterized in order to study their reaction with $^1O_2$ under different experimental conditions. 3β-Acetoxy- and 3β-mesyloxy-stigmasta-5,22-diene derivatives were also prepared and studied.

Introduction

The phototransformations of steroids owing to the direct absorption of light or to the interaction with electronic excited species have continuously called the attention of researchers. It is known that sterols as important lipidic components of human skin cells, may contribute to phototoxicity through photochemical reactions in the UVB and through oxygen dependent reactions in the UVA region.[1] Sterols with the 5,7,9-triene moiety are the only naturally occurring $^1O_2$ sensitizers described in the UVA region but they are also easily oxidized by this species. On the other hand, cholesterol and other sterols with a solitary double bond do not absorb UVA or UVB light, but quench $^1O_2$. In order to prevent photodamage owing to photooxidation of steroids the $^1O_2$ quenching process needs to be understood.

In an early work (1958), Schenck and co-workers showed that cholesterol reacts with $^1O_2$ yielding 5α-hydroperoxy-3β-hydroxycholest-6-ene which rearranges, in a non-polar solvent, to 7α-hydroperoxy-3β-hydroxycholest-5-ene.[2] In 1961, Nickon showed that hydroperoxidation of steroid monoolefins is stereospecific, and the new C–O bond bears a *cis* relationship to the C–H bond that is broken.[3a,b] In 1967, Smith *et al.* showed that 7α-hydroperoxy-3β-cholest-5-ene then underwent a slower epimerization to the 7β-hydroperoxide-3β-cholest-5-ene.[4] Since these early works a dozen or so further examples of this phenomenon in which an allyl hydroperoxide rearranges to its allylic isomer, have been identified, half of which refer to cholestene derivatives. It has been stated,[5] that this rearrangement occurs only when the initial and final hydroperoxide are substitutionally nonequivalent, and when the initial hydroperoxide has a composition of allylic isomers which is different from that of the equilibrium mixture resulting from rearrangement. Several years afterwards Beckwith (1989) *et al.*[6] showed with cholesterol, that the tertiary 5α-hydroperoxide originally formed rearranges by a non-dissociative mechanism (sigmatropic [2,3]-rearrangement; Schenck supra-facial rearrangement) yielding the 7α-hydroperoxide. The latter then undergoes a Smith epimerization through a dissociative mechanism yielding the 7β-hydroperoxide. Independently, Davies in 1989 demonstrated similar mechanisms for the rearrangements of allylic hydroperoxides derived from (+)-valencene.[7] In the former report,[6] the authors also described the photosensitized oxygenation of some 3-*O*-derivatives of cholesterol. Under the same conditions in which cholesterol gave the 5α-hydroperoxide, the 3-*O*-acetyl, the 3-*O*-methyl and the 3-*O*-trimethylsilyl derivatives gave principally the corresponding 7α- rather than the 5α-hydroperoxide. Besides, the authors observed that by treatment with $^1O_2$ epicholesterol, the 3α isomer of cholesterol, did not yield the corresponding 5- and the 7-hydroperoxides.

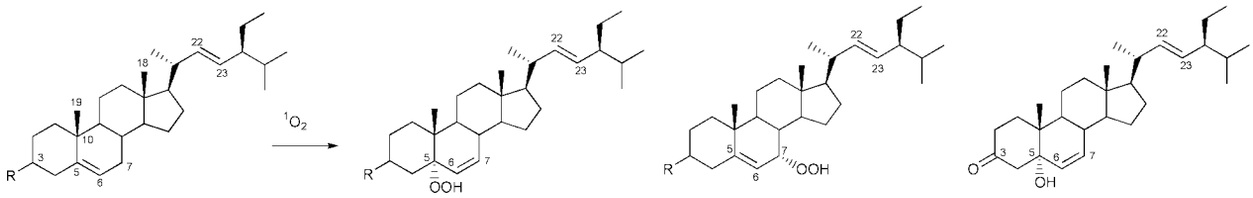
In an attempt to understand further some of the structural factors that affect the reactivity of steroids with $^1O_2$, we have examined the reaction of stigmasterol (Scheme 1, **1**), a 5,22-diene sterol, and some 3β-substituted stigmastadienes. Thus, 3β-F (**3**), 3β-Cl (**4**), 3β-Br (**5**), 3β-I (**6**) and 3α-Br (**8**) were firstly prepared and fully characterized in order to study their reaction with $^1O_2$ in different experimental conditions. 3β-Acetoxy and 3β-mesyloxy stigmastane derivatives, compounds **2** and **7** respectively, were also prepared and studied.

Results and discussion

In order to demonstrate that not only is the configuration at position 3 important to determine the formation and the reactivity of the 5α-hydroperoxide, but the chemical nature of the substituent at position 3 might also be important, we selected several 3β-substituted stigmasterols for photosensitized oxidation with Rose Bengal.

Stigmasterol (**1**) in pyridine containing Rose Bengal was stirred under oxygen and irradiated by a W lamp for 24 hours. The ^{1}H NMR and the ^{13}C NMR spectra showed the presence of the expected 5α-hydroperoxide **1a** together with the rearranged isomer 7α-hydroperoxide **1b** (Scheme 1, Table 1, δ_H **1**, 5.34 (1H, d, 6-H) and 7-H as part of complex signals at $\delta_H < 2.35$ ppm; **1a**, 5.75 (1H, d, 6-H) and 5.60 (1H, d, 7-H); **1b**, 5.73 (1H, br s, 6-H) and 4.09 (1H, m, 7-H). Table 2, δ_C **1**, 140.9 (5-C), 121.7 (6-C) and 31.9 (7-C); **1a**, 83.4 (5-C), 129.1 (6-C) and 129.2 (7-C); **1b**, 147.6 (5-C), 120.3 (6-C) and 77.9 (7-C)). Similar results were obtained when 3β-acetoxystigmasterol (**2**) was photooxidated in similar experimental conditions, the products being characterized as the 5α and 7α-hydroperoxide isomers **2a** and **2b** by

DOI: 10.1039/b000144i



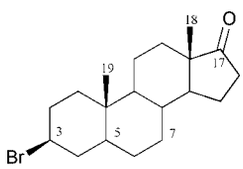
Steroid R	Products: 5α-Hydroperoxide 6,22-diene	7α-Hydroperoxide 5,22-diene	3-Keto 5-hydroxy 6,22-diene
1 β-HO	**1a**	**1b**	—
2 β-CH_3COO	**2a**	**2b**	—
3 β-F	—	**3b**	—
4 β-Cl	—	**4b**	—
5 β-Br	—	—	**5c**
6 β-I	—	—	**5c**
7 β-CH_3SO_3	—	—	**5c**
8 α-Br	—	—	—

Scheme 1 Stigmastadienes studied and products obtained.

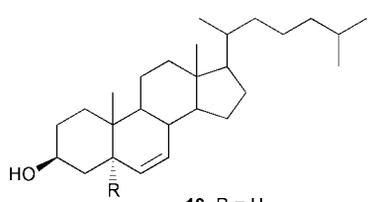
^{1}H and ^{13}C NMR spectra (Scheme 1, Tables 1 and 2, see the corresponding ^{1}H and ^{13}C NMR data of compounds **2**, **2a** and **2b**). It is interesting to note that both the ^{1}H NMR and the ^{13}C NMR spectra of **1a**, **1b**, **2a** and **2b** showed the presence of the unmodified double bond at 22-C/23-C position (Table 1, footnote (*a*) δ_H 4.90–5.25 (2H, m, 22-H and 23-H); Table 2, δ_c 134.3/129.6, 137.8/129.6, 135.1/129.4 and 138.1/129.4 ppm respectively). The regioselectivity observed in the Schenck ene reaction will be discussed further, in terms of molecular modelling.

When 3β-fluoro- (**3**) and 3β-chloro-stigmasterol (**4**) were oxygenated following the general method only the corresponding 7α-hydroperoxides **3b** and **4b** were obtained (Scheme 1, Tables 1 and 2, compare ^{1}H NMR data (6-H and 7-H) and ^{13}C NMR data (5-C, 6-C and 7-C) data for compounds **3**, **3b**, **4** and **4b**). It was previously proposed for cholesterol[8] that the intermolecular hydrogen bonding between the OH and the OOH groups would explain why when under conditions that the cholesterol reacted with singlet oxygen to give the unrearranged 5α-hydroperoxide, its acetyl derivative gave principally the rearranged 7α-hydroperoxide.[7] The fact that from **3** and **4** only the 7α-hydroperoxide was obtained agrees with this proposal.

When the 3β-bromostigmasta derivative **5** was irradiated according to the general conditions, the ^{1}H NMR spectrum of the only product obtained showed that this compound kept in its structure the 6-ene double bond (Scheme 1, **5c;** Table 1, **5c**, δ_H 5.63 (1 H, dd, 6-H) and 5.43 (1 H, dd, 7-H)) but had lost the methine (H–C) group at 3-C. The ^{13}C NMR spectrum confirmed the absence of an HCBr group at 3-C position (Table 2, compare 3-C data for **5** and **5c**) and the presence of the double bond at 6-C/7-C position (Table 2, 6-C and 7-C data for **5** and **5c**, δ_C 122.3/31.9 and 133.0/130.0 ppm respectively) together with a carbonyl group at 3-C (Table 2, **5c**, δ_C 190.4 ppm, C=O). The presence of the carbonyl group was also confirmed by the corresponding FTIR spectrum (KBr; ν 1715 cm^{-1}). Simultaneously the presence of the OH group instead of the OOH group at 5-C was confirmed by using chemical and spectroscopic tools. Thus, compound **5c** was recovered unchanged after treatment with reductive reagents such as triphenylphosphine as was confirmed by FTIR, MS, ^{1}H and ^{13}C NMR spectra; under the same experimental conditions the 3β-hydroxy-5α-hydroperoxycholest-6-ene (**10a**) yielded the corresponding 3β,5α-dihydroxycholest-6-ene (**10c**), and the 3β-hydroxy-7α-hydroperoxycholest-5-ene (**10b**) yielded the corresponding 3β,7α-dihydroxycholest-5-ene (**10d**), in agreement with results that had been previously reported.[6]

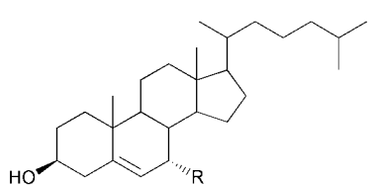


9
3β-Bromo-5α-androstan-17-one



10 R = H
10a R = OOH

10c R = OH



10b R = OOH

10d R = OH

When the photooxygenation of (22*E*)-3β-iodostigmasta-5,22-diene **6** and (22*E*)-3β-mesyloxystigmasta-5,22-diene **7** were carried out in the same experimental conditions we obtained in each case, as the only product, a compound that was characterized by TLC, FTIR, MS, ^{1}H and ^{13}C NMR spectra as compound **5c** (Scheme 1).

When the photosensitized oxygenations of **5**, **6** and **7** were carried out in the presence of galvinoxyl (*ca.* 10 mol%), a known radical inhibitor that should inhibit any possible radical chain reaction of the primary hydroperoxide product, the corresponding 5α-hydroperoxy-3β-bromo-, 5α-hydroperoxy-3β-iodo- and 5α-hydroperoxy-3β-mesyloxystigamasta-6,22-diene were not obtained. In each case, the 3-keto-5α-hydroxystigmasta-6,22-diene **5c** was detected as the only product. In an additional experiment we observed that when the oxygenation of 3β-bromostigmasta **5** was carried out in pyridine with a high H_2O_2 concentration (*ca.* 1000:1 mol:mol) the 3-keto product

Table 1 Partial ^{1}H NMR spectra of the stigmasta studied and the photooxidated products obtained

H	**1**[a]	**1a**[a]	**1b**[a]	**2**[a,b]	**2a**[a,b]	**2b**[a,b]	**3a**[a]	**3b**[a]	**4a**[a]	**4b**[a]	**5a**[a]	**6**	**7**	**5c**
3-H	3.51	4.07	3.75	4.60	4.70	4.70	4.40	4.46	4.48	4.15	3.95	4.04	4.52	—
	(m, 1H)	(m, 1H)	(m, 1H)	(m, 1H)	(m, 1H)	(m, 1H)	(d, *J* 50, 1H)	(d, *J* 50, 1H)	(m, 1H)	(m, 1H)	(m, 1H)	(m, 1H)	(m, 1H)	
6-H	5.34	5.75	5.73	5.40	5.74	5.75	5.40	5.76	5.34	5.73	5.38	5.32	5.40	5.63
	(d, *J* 5, 1H)	(d, *J* 9, 1H)	(br s, 1H)	(d, *J* 5, 1H)	(d, *J* 9, 1H)	(br s, 1H)	(d, *J* 5, 1H)	(d, *J* 5, 1H)	(d, *J* 5, 1H)	(d, *J* 5, 1H)	(d, *J* 5, 1H)	(d, *J* 5, 1H)	(d, *J* 5, 1H)	(dd, *J* 3 and 10, 1H)
18-CH_3	0.70	0.85	0.80	0.72	0.82	0.78	0.72	0.82	0.70	0.68	0.74	0.70	0.75	0.69
	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 1H)	(s, 3H)	(s, 3H)	(s, 3H)
19-CH_3	1.00	1.02	1.00	1.04	1.00	1.00	1.05	1.05	1.04	1.02	1.10	1.0	1.05	1.00
	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)	(s, 3H)
7-H	— [c]	5.60	4.09	— [c]	5.60	4.13	— [c]	4.16	— [c]	3.85	— [c]	— [c]	— [c]	5.43
		(d, *J* 9, 1H)	(m, 1H)		(d, *J* 9, 1H)	(m, 1H)		(dd, *J* 3 and 5, 1H)		(m, 1H)				(dd, *J* 3 and 10, 1H)

J Values are given in Hz. [a] 4.90–5.25 (2H, m, 22-H and 23-H). [b] 2.40 (3H, s, CH_3COO). [c] As part of complex signals at $\delta < 2.35$ ppm.

5c was not detected in the reaction medium; a similar result was obtained when the experiment was conducted in dichloromethane instead of pyridine. The results obtained from the three above mentioned experiments suggest that the oxidative substitution at 3-CBr to give 3-C=O must occur through an intramolecular non-radical reaction.

When the isomer of (22*E*)-3β-bromostigmasta-5,22-diene (**5**), the (22*E*)-3α-bromostigmasta-5,22-diene **8** was submitted to photooxygenation neither the (22*E*)-3α-bromo-5α-hydroperoxystigmasta-6,22-diene nor the (22*E*)-3α-bromo-7α-hydroperoxystigamasta-5,22-diene or the (22*E*)-5α-hydroxystigmasta-6,22-dien-3-one **5c** were obtained.

In order to study the stability of the Br–C σ bond at the 3β position in the presence of $^1O_2$, we prepared a 3β-bromo steroid analogue to compound **5** which does not have the 3-C in an allylic moiety (double bond at 1-C or at 4-C). Thus, 3β-bromo-5α-androst-17-one **9** was prepared according to known methods. Irradiated in the presence of Rose Bengal and oxygen in pyridine solution, compound **9** was recovered unchanged. The hydroperoxidation of the double bond at 5-C is the necessary previous step in the formation of **5c**.

These results favor a three-step mechanism for the reactions studied (Scheme 2). The first and the second steps agree with

Scheme 2

the Schenck ene reaction:[2,6,7] in the former step an exciplex with perepoxide-like geometry is formed[9,10] and in the latter the allylic hydrogen atom at 7-C is abstracted to afford the 5α-hydroperoxide with the double bond at the 6-C/7-C position (Scheme 2, steps a and b).[11] From this 5α-hydroperoxide, instead of the well known sigmatropic [2,3]-rearrangement to the 7α-hydroperoxide with the double bond at 5-C/6-C position (Scheme 2, step c), an intramolecular S_N^2 mechanism involving the 5-C–OOH and 3-CH–Br groups occurs yielding the 3,5-endoperoxide (Scheme 2, step d). In turn cleavage of the O–O

Table 2 ^{13}C NMR spectra of the stigmasta studied and the photooxidated products obtained

C	1	1a	1b	2	2a	2b	3	3b	4	4b	5	6	7[a]	5c
1	37.5	36.8	38.9	37.0	38.2	38.1	39.3	39.1	39.2	38.6	39.7	39.6	39.2	30.1
2	31.7	28.5	35.3	27.7	28.3	28.9	36.4	38.7	33.5	33.1	34.5	36.6	36.9	37.5
3	71.8	66.3	70.7	73.8	86.7	83.6	92.8	92.2	59.9	59.3	52.5	31.9	82.0	190.4
4	42.4	39.7	41.8	38.1	39.7	42.2	28.7	28.4	43.5	43.3	44.4	46.4	39.6	49.1
5	140.9	83.4	147.6	139.4	73.5	147.5	139.4	147.2	140.7	148.4	141.5	142.8	138.6	75.1
6	121.7	129.1	120.3	122.4	129.3	121.0	123.1	121.2	122.4	120.6	122.3	121.7	123.8	133.0
7	31.9	129.2	77.9	31.8	129.0	78.1	31.9	78.3	30.8	78.3	31.9	31.8	31.9	130.0
8	31.9	38.8	37.0	31.8	31.9	31.9	31.93	37.0	32.3	37.0	31.9	30.4	31.8	37.9
9	50.3	43.7	43.4	50.0	49.0	49.0	50.1	43.4	50.1	43.5	50.3	50.5	50.0	44.8
10	36.6	38.7	36.6	36.5	36.5	37.0	29.7	37.4	36.6	37.3	36.5	36.5	36.4	37.9
11	21.1	20.7	20.7	21.0	21.0	21.0	21.2	20.9	21.3	20.7	21.2	20.8	21.1	21.0
12	39.8	30.8	29.9	39.6	27.5	26.4	39.7	39.6	39.6	38.9	40.5	41.9	29.0	39.8
13	42.4	43.2	43.2	42.2	43.5	43.5	42.2	42.2	42.3	42.2	42.3	42.4	42.2	40.0
14	57.0	51.2	48.9	56.7	55.7	56.0	56.8	49.1	56.8	49.1	56.9	56.8	56.8	53.0
15	24.4	23.8	24.3	24.3	23.8	24.4	24.4	24.4	24.4	24.4	24.4	24.3	24.4	23.9
16	28.9	28.7	28.9	28.9	28.8	28.8	29.0	28.8	29.0	28.9	29.0	28.9	28.9	28.9
17	56	53.6	55.7	55.9	53.5	55.7	56	55.6	56.0	55.6	56.1	56.0	56.0	55.0
18	12.2	11.3	12.1	12.0	11.5	12.2	12.3	12.2	12.4	12.2	12.3	12.3	12.3	12.3
19	19.4	15.1	18.0	19.3	15.2	18.1	19.0	18.1	19.1	18.1	19.1	19.0	19.3	14.0
20	40.5	40.4	40.4	40.5	40.4	40.4	40.5	40.3	40.5	40.4	40.5	40.1	40.5	40.4
21	21.1	21.0	21.0	21.3	21.3	21.3	21.3	21.2	21.0	21.0	21.3	21.1	21.3	21.2
22	138.4	134.3	137.8	138.1	135.1	138.1	138.2	138.1	138.2	138.1	138.1	138.3	138.2	138.0
23	129.4	129.6	129.6	129.1	129.4	129.4	129.4	129.3	129.2	129.4	129.4	129.4	129.4	129.5
24	51.3	51.2	51.2	51.2	51.2	51.2	51.3	51.2	51.2	51.2	51.3	51.2	51.3	51.3
25	31.9	31.8	31.8	31.8	31.8	31.8	31.9	31.9	31,8	31.9	31.9	31.7	31.8	31.8
26	19.0	18.8	18.8	19.0	19.0	19.0	19.3	18.1	19.4	19.0	19.3	19.3	19.0	19.3
27	21.1	20.8	20.8	21.0	21.0	21.0	21.1	21.0	21.3	21.2	21.1	21.3	21.1	21.3
28	25.4	25.2	25.2	25.4	25.4	25.4	25.4	25.5	25.5	25.4	25.5	25.4	25.4	25.4
29	12.0	12.0	12.0	12.2	11.5	12.2	12.2	12.1	12.1	11.5	12.2	12.1	12.1	12.2

[a] CH_3 (mesyloxy group) 38.8 ppm.

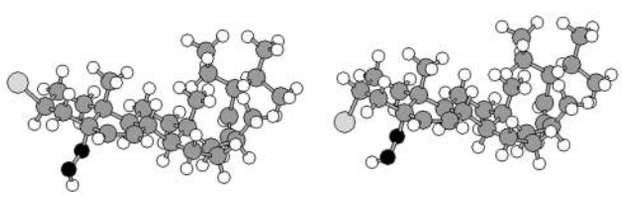
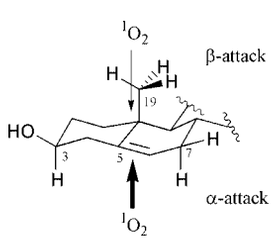


Fig. 1 Optimized structure of 3β-bromo- (left) and 3α-bromo- (right) 5α-hydroperoxystigmasta-6,22-diene (Scheme 1, compounds **5** and **8**, respectively).

bond, by a well known reaction,[12] gives a more stable oxygenated structure containing both the 5-C–OH and the 3-C=O groups (Scheme 2, 5α-hydroxy-3-keto derivative; Scheme 1, **5c**). The presence of good leaving groups such as Br-, I-, mesyloxy- at 3-C and the easy approach of the 5-C–OOH group at 3-C shown in Fig. 1, would account for the results obtained.

Stereoselectivity and regioselectivity

The α-π-facial stereoselectivity of the reaction of singlet oxygen with cholesterol to give 5α-hydroperoxycholest-6-en-3β-ol is well accepted owing to the fact that the methyl group at 10-C is β (axial) (Scheme 3, 19-CH_3).[2–8]



Scheme 3

Thus, as expected the photosensitized oxidation of stigmasterol **1** in pyridine with Rose Bengal yielded the 5-hydoperoxide **1a** which undoubtedly is, according to spectroscopic data, the 5α-isomer. AM1 and *ab initio* HF/3-21G calculations support these experimental results. The calculated activation energy for stigmasterol–$^1O_2$ exciplex formation for the $^1O_2$ α-attack is lower than that for the β analogue (Scheme 2, step a).

When the isomer of 3β-bromostigmasta-5,22-diene, the 3α-bromostigmasta-5,22-diene (**8**) in which the group at position 3 is α (axial) was submitted to the same treatment, no reaction was observed. Fig. 1 depicts the AM1 calculated structure of 3β-bromo- and 3α-bromo-5α-hydroperoxy-stigmasta-6,22-diene used for calculations ($\Delta H_f = -26.5164$ and -18.0654 kcal mol^{-1} respectively), in which the 1,3-diaxial disposition of Br and OOH groups in the latter compound is clearly displayed. For this isomer the atomic charges values calculated (AM1) for the 3α-Br and for the O atoms of the 5α-HOO group are -0.170, -0.204 and -0.135 respectively. As consequence the stereoelectronic repulsive effect is so important that the primary photooxygenated product, 5α-hydroperoxy-derivative would not be formed.

As was mentioned above, when the 3-keto compound **5c** was obtained by photooxygenation of **5**, the substitution of the Br group in the 3β-bromostigmasta molecule occurs from the 5α-hydroperoxide intermediate through an intramolecular S_N^2 reaction. As can be seen in Fig. 1, the distance between the hydroperoxide group at 5-C and the 7-C and between the same hydroperoxide group and at 3-C are equivalent. Thus, instead of the well known intramolecular 5 to 7 hydroperoxide concerted rearrangement (*via* a 5,7-endoperoxide like intermediate; Scheme 2, step c) an intramolecular nucleophilic substitution *via* a 3,5-endoperoxide-like intermediate occurs (Scheme 2, step d). The presence of excellent leaving groups such as Br (**5**), I (**6**) and mesyloxy (**7**) at 3-C favors the course of the reaction through the proposed 3,5-endoperoxide intermediate, this intramolecular rearrangement being faster than any other competitive intramolecular rearrangement, and particularly faster than the 5,7-rearrangement mentioned above.

As was pointed out, in all the products obtained the presence of the unmodified double bond at the 22-C/23-C position was easily confirmed by NMR. The clear regioselectivity of the

singlet oxygen attack of the 5-C/6-C double bond was a bit surprising. Analysis of the geometry optimised structures (AM1 and *ab initio* (HF/3-21G) level) showed that the $^1O_2$ attack of the 22-C/23-C double bond is feasible from a steric point of view. In addition the HOMO's of stigmastas **1**–**8** were calculated (ZINDO/S//AM1 and ZINDO/S//HF/3-21G level). The shape of the calculated orbitals is almost the same for all the compounds, the largest coefficients were found at the 5-C/6-C double bond and no important (or any) contribution of the 22-C/23-C π-system to the HOMOs was observed. As the $^1O_2$ reaction on 5-C/6-C double bond can be considered an electrophilic attack forming an exciplex with perepoxide-like geometry in the rate determining path,[9] (Scheme 2, step a), the analysis of the HOMO's properties of the stigmasta studied agrees with the regioselectivity observed.

Conclusion

This paper demonstrates that in the photooxidation of 3β-substituted stigmasterols with $^1O_2$ not only is the configuration at 3-C important to determine the formation and the reactivity of the 5α-hydroperoxide derivatives, but the chemical nature of the substituent at 3-C is also very important. Thus, depending on the nature of this substituent, an alternative pathway for the classical Schenck rearrangement can occur.

Experimental

Reagents and solvents

Melting points were determined on a Fisher Jones apparatus and were not corrected. IR spectra were recorded on a Nicolet-Magna-550-FTIR Spectrophotometer. ^1H NMR and ^{13}C NMR spectra were registered on a Bruker AC-200 spectrometer, chemical shifts (δ) are reported in parts per million (ppm), relative to internal tetramethylsilane. *J* values are given in Hz. The measurements were carried out using the standard pulse sequences. Electron impact mass spectra (EI-MS) and GC-MS analyses were recorded on a VG-TRIO-2 mass spectrometer attached to a Hewlett Packard 5890 gas chromatograph; high resolution mass spectra (HR-MS) and fast atom bombardment mass spectra (FAB-MS) were recorded on a VG-ZAB BEQ instrument. Column chromatography was carried out on Merck silica gel 60 (0.040–0.063 mm) and TLC on Merck silica gel 60 F-254 (aluminum sheets, 0.2 mm thickness). Toluene, hexane, methylene chloride, chloroform, ether and ethyl acetate of analytical grade were used after purification. Carbon tetrabromide and triphenylphosphine were purchased from Aldrich. Acetonitrile, Na_2SO_4, $NaHCO_3$ and pyridine were obtained from JT Baker. 48% Aqueous HF from La Fluorhidrica. 37% Aqueous HCl, 37% aqueous HBr and 50% aqueous HI were obtained from Merck. Rose Bengal (sodium salt 95%), stigmasterol (**1**) and cholesterol (**10**) were purchased from Sigma. Androsterone was purchased from Aldrich.

(22*E*)-3α,5-Cyclo-6β-hydroxy-5α-stigmast-22-ene,[13] (22*E*)-3β-acetoxystigmasta-5,22-diene (**2**)[14] and (22*E*)-3β-mesyloxy-stigmasta-5,22-diene (**7**)[14] were prepared according to well established procedures and were characterized by their spectral data.

Synthesis of steroids

(22*E*)-3β-Fluorostigmasta-5,22-diene 3. To a solution of 205 mg (0.5 mmol) of (22*E*)-3α,5-cyclo-6β-hydroxy-5α-stigmast-22-ene in 20 ml of toluene, 10 ml of 48% aqueous HF was added, and the resulting mixture was stirred for 30 minutes at room temperature. The organic phase was separated, washed first with a saturated solution of $NaHCO_3$ and then with water, dried over Na_2SO_4, and the solvent evaporated under vacuum. The resulting solid residue was purified by flash column chromatography through silica gel with hexane as eluent. Pure (22*E*)-3β-fluorostigmasta-5,22-diene (**3**) was obtained in 70% yield; mp 114–115 °C; ν_{max}(film)/cm^{-1} 1641 (s), 1472 (w), 1387 (w), 1018 (w), 980 (w); EI-MS, *m*/*z*: 414 ($M^{+\bullet}$, 12.2%), 399 ($M^{+\bullet}$ − 15, 1.4), 371 ($M^{+\bullet}$ − 43, 4.9); Found: 414.3659, Calc. for $C_{29}H_{47}F$: 414.3662.

(22*E*)-3β-Chlorostigmasta-5,22-diene 4. To a solution of 185 mg (0.45 mmol) of (22*E*)-3α,5-cyclo-6β-hydroxy-5α-stigmast-22-ene in 18 ml of toluene, 10 ml of 37% aqueous HCl were added following the synthesis method described above. Pure (22*E*)-3β-chlorostigmasta-5,22-diene **4** was obtained in 73% yield; mp 89–90 °C (lit.[14] 89–90 °C); ν_{max}(film)/cm^{-1} 1648 (s), 1472 (w), 1387 (w), 972 (s), 765 (s); EI-MS, *m*/*z*: 432 ($M^{+\bullet}$, ^{37}Cl, 3.58%), 430 ($M^{+\bullet}$, ^{35}Cl, 8.3); Found: 430.3366, Calc. for $C_{29}H_{47}Cl$: 430.3366.

(22*E*)-3β-Bromostigmasta-5,22-diene 5. To a solution of 240 mg (0.58 mmol) of (22*E*)-3α,5-cyclo-6β-hydroxy-5α-stigmast-22-ene in 24 ml of toluene, 12 ml of 37% aqueous HBr were added following the synthesis method described above. Pure (22*E*)-3β-bromostigmasta-5,22-diene **5** was obtained in 73% yield; mp 100–101 °C (lit.[14,15] 100–101 °C); ν_{max}(film)/cm^{-1} 1645 (s), 1460 (w), 1370 (w), 980 (s), 790 (w); EI-MS, *m*/*z*: 476 ($M^{+\bullet}$, ^{81}Br, 3.9%), 474 ($M^{+\bullet}$, ^{79}Br, 4.4); Found: 474.2862, Calc. for $C_{29}H_{47}Br$: 474.2861.

(22*E*)-3β-Iodostigmasta-5,22-diene 6. To a solution of 2.7 g (6.55 mmol) of (22*E*)-3α,5-cyclo-6β-hydroxy-5α-stigmast-22-ene in 60 ml of benzene, 1 ml of 50% aqueous HI were added following the synthesis method described above. Pure (22*E*)-3β-iodostigmasta-5,22-diene **6** was obtained in 72% yield; mp 98–99 °C; ν_{max}(film)/cm^{-1} 1667 (w), 1463 (s), 1455 (w), 1387 (s), 955 (s), 750 (w); EI-MS, *m*/*z*: 522 ($M^{+\bullet}$, 1.35%), 395 (68.8); Found; 522.2719, Calc. for $C_{29}H_{47}I$: 522.2723.

(22*E*)-3α-Bromostigmasta-5,22-diene 8. A mixture of stigmasterol (**1**, 1 g, 2.42 mmol) in 32 ml of chloroform, carbon tetrabromide (5 g) and triphenylphosphine (3.7 g) was stirred at room temperature for two hours. The resulting product was filtered through silica gel using ethyl acetate as eluent and then recovered after the solvent was evaporated under vacuum. The crude product was purified by column chromatography using hexane as eluent (54 mg, 5%) to give a white powder; mp 88–89 °C (lit.[15] 90 °C); $\delta_H(CDCl_3)$ 0.7 (3H, s, Me-18), 1.0 (3H, s, Me-19), 4.67 (1H, m, H-3), 5.37 (1H, br s, H-6); EI-MS, *m*/*z*: 476 ($M^{+\bullet}$, ^{81}Br, 2%), 474 ($M^{+\bullet}$, ^{79}Br, 2);[15] Found: 474.2862, Calc. for $C_{29}H_{47}Br$: 474.2861.[15]

3β-Bromo-5α-androstan-17-one 9. A mixture of androsterone (1 g, 3.44 mmol) in 80 ml of chloroform, carbon tetrabromide (7 g) and triphenylphosphine (5.5 g) were stirred at room temperature for 3 hours. The resulting product was filtered through silica gel using hexane–ethyl acetate as eluent (1 : 1) and then recovered after the solvent was evaporated under vacuum. The crude product was purified by column chromatography using hexane–ethyl acetate as eluent (800 mg, 80%); mp 144–146 °C (lit.[16] 147 °C); $\delta_H(CDCl_3)$ 0.85 (3H, s, Me-18), 0.87 (3H, s, Me-19), 4.02 (1H, m, H-3); $\delta_C(CDCl_3)$ 12.2 (C-18), 13.7 (C-19), 20.2 (C-11), 21.6 (C-15), 21.7 (C-17), 28.0 (C-6), 30.6 (C-7), 31.4 (C-12), 33.9 (C-2), 34.8 (C-8), 35.4 (C-10), 35.7 (C-16), 39.6 (C-1), 40.4 (C-4), 47.6 (C-13), 47.8 (C-5), 51.3 (C-9), 51.9 (C-14), 54.2 (C-3).

Photochemical reactions

A solution of the steroid (1 g) and Rose Bengal (10 mg) in pyridine (10 ml) contained in a Pyrex tube kept in a water-cooled bath was saturated with oxygen. The solution which was vigorously stirred by the gas bubbling, was irradiated with light from a 220 V 400 Im-CE 4 300 W lamp placed at a distance of 15 cm. The oxygen bubbling was ceased after 24 hours. The pyridine was removed under reduced pressure and the solid

residue was dissolved in a minimum volume of methylene chloride and adsorbed on silica gel. Then the silica gel was added to the top of a preparative silica flash chromatography column and eluted with the appropriate solvent mixtures in each case.

Irradiations in CH_3CN, CH_3CN–Cl_2CH_2, Cl_2CH_2, EtOH and CH_3CN–pyridine media were performed according to the above protocol. Thus, solutions of 50 mg of stigmasterol **1** in 50 ml of the selected solvent and 1 mg of photosensitizer were irradiated. In all the cases, the corresponding 7α-hydroperoxide derivative was obtained in low yield. After isolation of the product obtained, the ^{1}H NMR spectrum obtained was similar to that described for **1b**. Better results were obtained when irradiations were performed in the presence of pyridine.

(22*E*)-7α-Hydroperoxy-3β-hydroxystigmasta-5,22-diene 1b. A solution of stigmasterol (**1**, 995 mg) and Rose Bengal (10 mg) in pyridine (10 ml) was irradiated according to the general technique. The reaction mixture was separated by preparative silica gel flash chromatography eluted with diethyl ether–hexane (1 : 1) to give 457 mg (58%) of the hydroperoxide **1b** as a white powder; mp 136–138 °C; ν_{max}(film)/cm^{-1} 3438(s), 1650 (m), 1269 (m), 1061 (m), 749 (s); EI-MS, *m*/*z*: 426 ($M^{+\bullet} - H_2O$, 1.4%), 410 ($M^{+\bullet} - H_2O_2$, 4.8), 55 (100); FAB, *m*/*z*: 428 ($M^{+\bullet}$ − O, 19%), 427 ($M^{+\bullet}$ − OH, 100), 412 ($M^{+\bullet}$ − 20, 15), 411 ($M^{+\bullet}$ − OOH, 95), 393 ($M^{+\bullet}$ − OOH − H_2O, 45).

(22*E*)-3β-Acetoxy-7α-hydroperoxystigmasta-5,22-diene 2b. A solution of (22*E*)-3β-acetoxystigmasta-5,22-diene (**2**, 863 mg) and Rose Bengal (8 mg) in pyridine (10 ml) was irradiated according to the general technique. The reaction mixture was purified by preparative silica gel flash chromatography eluted with diethyl ether–hexane (1 : 1) giving 96 mg (12%) of the hydroperoxide **2b** as a white powder; mp 83–84 °C; ν_{max}(film)(cm^{-1}): 3382 (b), 1739 (s), 1466 (s), 1378 (s), 1257 (s), 1033 (s), 985 (m), 744, 608; EI-MS *m*/*z*: 470 (M − O, 1%); 468 (M − H_2O, 2.2); 453 (M − HO_2, 1.5), 426 (M − CH_3COOH, 5).

(22*E*)-3β-Fluoro-7α-hydroperoxystigmasta-5,22-diene 3b. A solution of (22*E*)-3β-fluorostigmasta-5,22-diene (**3**, 121 mg) and Rose Bengal (1 mg) in pyridine (10 ml) was irradiated according to the general technique. The reaction mixture was purified by preparative silica gel flash chromatography as usual to give 38.3 mg (31%) of the hydroperoxide **3b** as a syrup, that could not be crystallized; ν_{max}(film)/cm^{-1} 3392, 1663, 1418, 1369, 980, 953, 734; EI-MS, *m*/*z*: 428 ($M^{+\bullet}$ − 18, 5.4%), 412 ($M^{+\bullet} - H_2O_2$, 1.4); FAB-MS, *m*/*z*: 430 ($M^{+\bullet}$ − O, 7.0%), 429 ($M^{+\bullet}$ − OH, 100).

(22*E*)-3β-Chloro-7α-hydroperoxystigmasta-5,22-diene 4b. A solution of (22*E*)-3β-chlorostigmasta-5,22-diene (**4**, 300 mg) and Rose Bengal (3 mg) in pyridine (5 ml) was irradiated according to the general technique. After the reaction mixture was purified by preparative silica gel flash chromatography as usual to give 47 mg (16%) of the hydroperoxide **4b** as a colourless syrup, that could not be crystallized; ν_{max}(film)/cm^{-1} 3446, 1658, 1466 1394, 1273, 977, 881, 752; EI-MS, *m*/*z*: 464 ($M^{+\bullet}$, ^{37}Cl, 0.36%), 462 ($M^{+\bullet}$, ^{35}Cl, 0.84), 448 ($M^{+\bullet}$ − O, 2.8), 446 ($M^{+\bullet}$ − O, 8.9), 431 ($M^{+\bullet}$ − HO_2, 1.0), 429 (M − H_2O_2, 2.1); FAB-MS, *m*/*z*: 446 ($M^{+\bullet}$ − O, 0.25%), 445 ($M^{+\bullet}$ − OH, 100), 409 ($M^{+\bullet}$ − H_2O − Cl, 33).

(22*E*)-5α-Hydroxystigmasta-6,22-dien-3-one 5c. A solution of (22*E*)-3β-bromostigmasta-5,22-diene (**5**, 714 mg) and Rose Bengal (10 mg) in pyridine (10 ml) was irradiated according to the general technique. The reaction mixture obtained was purified by preparative silica gel flash chromatography, eluted with mixtures of hexane–ethyl acetate to give 98 mg (17%) of the (22*E*)-5α-hydroxystigmasta-6,22-dien-3-one **5c** as a white powder; mp 178–180 °C; ν_{max}(film)/cm^{-1} 1715, 1383, 973, 950, 748; EI-MS, *m*/*z*: 426 ($M^{+\bullet}$, 0.9%); 408 ($M^{+\bullet}$ − H_2O, 4); FAB-MS, *m*/*z* 426 (M, 3%); 425 (M − H, 22); 409 ((M − OH, 100), Found: 426.3492, Calc. for $C_{29}H_{46}O_2$: 426.3498.

Similar results were obtained when the reaction was carried out in the presence of galvinoxyl (*ca*. 10%).

Similar results were obtained when the reaction was carried out with (22*E*)-3β-iodo- and (22*E*)-3β-mesyloxy-stigmasta-5,22-diene (compounds **6** and **7** respectively). Thus, **5c** (45 mg, 10%) was obtained when a solution with 450 mg of (22*E*)-3β-mesyloxy-stigmasta-5,22-diene **7** and Rose Bengal (10 mg) in pyridine (10 ml) was irradiated and purified according to the general method. The same product was obtained (60 mg, 5%), when a solution of 1.1 g of (22*E*)-3β-iodostigmasta-5,22-diene **6** and Rose Bengal (9.5 mg) in pyridine (10 ml) was irradiated.

Treatment of 5c with triphenylphosphine. A mixture of (22*E*)-5α-hydroxystigmasta-6,22-dien-3-one (**5c**) in 10 ml of ether, and an excess of triphenylphosphine was stirred at room temperature for 3 hours. Simultaneously 3β-hydroxy-5α-hydroperoxycholest–6-ene (**10a**) and 3β-hydroxy-7α-hydroperoxycholest-5-ene (**10b**) independently, were submitted to the same treatment. Compound **5c** was recovered unchanged and characterized by spectroscopic data (FTIR, MS, ^{1}H and ^{13}C NMR) while compound **10a** was completely converted to 3β,5α-dihydroxycholest-6-ene (**10c**) and compound **10b** was converted to 3β,7α-dihydroxycholest-5-ene (**10d**) (see experimental details below).

Reaction of (22*E*)-3β-bromostigmasta-5,22-diene (5) with hydrogen peroxide. Hydrogen peroxide 60% (2.7 g) was added to a solution of (22*E*)-3β-bromostigmasta-5,22-diene **5** in pyridine (1 ml) and the mixture was stirred in the dark for 24 hours. After this, half of the total volume was diluted with ethanol. Both, the pyridine and the ethanol solutions together with an authentic sample of (22*E*)-5α-hydroxystigmasta-6,22-dien-3-one **5c** were compared by TLC analysis. In these experiments, **5c** was not detected as reaction product.

A similar result was obtained when the experiment was conducted in dichloromethane instead of pyridine.

3β-Hydroxy-5α-hydroperoxycholest-6-ene 10a.[6] A solution of cholesterol (**10**, 300 mg) and Rose Bengal (3 mg) in pyridine (5 ml) was irradiated according to the general technique. The reaction mixture was purified by preparative silica gel flash chromatography yielding 205 mg (70%) of the hydroperoxide **10a** as white crystals from aqueous methanol; mp 142–143 °C (decomp.) (lit.[6] 141–142 °C (decomp.)); δ_H($CDCl_3$) 0.70 (3H, s, Me-18), 4.10 (m, 1H, 3-H), 5.60 (dd, 1H, 7-H, *J* = 9.90 and 2.70 Hz), 5.83 (dd, 1H, 6-H, *J* = 9.98 and 2.68 Hz); FAB-MS, *m*/*z*: 418 ($M^{+\bullet}$ − O, 7.0%), 400 ($M^{+\bullet}$ − H_2O, 100).

3β-Hydroxy-7α-hydroperoxycholest-5-ene 10b.[6] A solution of 3β-hydroxy-5α-hydroperoxycholest-6-ene (**10a**, 100 mg) in chloroform (5 ml) was kept in the dark at room temperature. After 24 hours the solvent was removed under reduced pressure. The reaction mixture was purified by preparative silica gel flash chromatography column yielding 85 mg (85%) of the rearranged 3β-hydroxy-7α-hydroperoxycholest-5-ene **10b** as white crystals from hexane–ether; mp 151–152 °C (lit.[6] 152–153 °C); δ_H($CDCl_3$) 0.67 (3H, s, Me-18), 3.71 (m, 1H, 3-H), 4.20 (m, 1H, 7-H), 5.69 (dd, 1H, 6-H, *J* = 5.00 and 1.84 Hz); FAB-MS, *m*/*z*: 418 ($M^{+\bullet}$ − O, 7.0%), 400 ($M^{+\bullet}$ − H_2O, 100).

3β,5α-Dihydroxycholest-6-ene 10c.[6] A solution of 3β-hydroxy-5α-hydroperoxycholest-6-ene (**10a**) (50 mg) in ether (10 ml) was reduced with triphenylphosphine according to the method described by Beckwith *et al.*[6] The reaction mixture was purified by preparative silica gel flash chromatography. 3β,5α-Dihydroxycholest-6-ene was obtained as white crystals from aqueous methanol, mp 158–160 °C; δ_H($CDCl_3$)[6] 0.68 (3H, s,

Me-18), 4.12 (m, 1H, 3-H), 5.56 (dd, 1H, 6-H, J = 9.85 and 2.57 Hz), 5.62 (dd, 1H, 7-H, J = 9.85 and 1.68 Hz); FAB-MS, m/z: 402 ($M^{+\bullet}$ − O, 7.0%), 385 ($M^{+\bullet}$ − HO, 100).

3β,7α-Dihydroxycholest-5-ene 10d.[6] A solution of 3β,7α-dihydroperoxycholest-5-ene (**10b**) (50 mg) in ether (10 ml) was reduced with triphenylphosphine according to the method described by Beckwith *et al.*[6] After the reaction mixture was purified by preparative silica gel flash chromatography column 3β,7α-dihydroxycholest-5-ene was obtained as white crystals from aqueous methanol; mp 160–162 °C; $\delta_H(CDCl_3)$[6] 0.69 (3H, s, Me-18), 3.57 (m, 1H, 3-H), 3.88 (m, 1H, 7-H), 5.62 (dd, 1H, 6-H, J = 5.30 and 1.67 Hz); FAB-MS, m/z: 402 ($M^{+\bullet}$ − O, 10%), 385 ($M^{+\bullet}$ − HO, 100).

Computational details

The ground state geometry and the heat of formation of the steroids were calculated (1) by using the AM1 method and (2) transfering the optimized geometries obtained by *ab initio* calculations (3-21G basis at HF level; Gaussian 94 interface; Cerius[2] 3.5, in a SiliconGraphics Octane™) by using the AM1 method. Molecular orbitals were calculated by using the ZINDO/S (CI, 6:6) method. A similar HOMO and LUMO description was obtained from geometries optimised at AM1 and at HF/3-21G levels.

Acknowledgements

We thank UBA and CONICET for partial financial support and UMYMFOR (FCEyN-UBA-CONICET) for technical support. EGG and REB are research members of CONICET.

References

1 W. Albro, P. Bilski, J. T. Corbett, J. L. Schroeder and C. F. Chignell, *Photochem. Photobiol.*, 1997, **66**, 316.
2 G. O. Schenck, O. A. Neumuller and W. Eisfeld, *Justus Liebigs Ann. Chem.*, 1958, **618**, 202.
3 (*a*) A. Nickon and J. F. Bagli, *J. Am. Chem. Soc.*, 1961, **83**, 1498; (*b*) A. Nickon and W. L. Mendelson, *J. Am. Chem. Soc.*, 1963, **85**, 1849.
4 L. L. Smith, W. S. Mathews, J. C. Price, R. C. Bachman and B. Reynolds, *J. Chromatogr.*, 1967, **27**, 187.
5 R. W. Denny and A. Nickon, *Org. React.*, 1973, **20**, 133.
6 A. L. J. Beckwith, A. G. Davies, I. G. E. Davison, A. Maccoll and M. H. Mruzek, *J. Chem. Soc.*, *Perkin Trans. 2*, 1989, 815.
7 A. G. Davies and I. G. Davison, *J. Chem. Soc.*, *Perkin Trans. 2*, 1989, 825.
8 G. O. Schenck, *Angew. Chem.*, 1957, **69**, 579.
9 W. Adam, C. R. Saha-Möller, S. B. Schembony, K. S. Schmid and T. Wirth, *Photochem. Photobiol.*, 1999, **70**, 476.
10 A. A. Frimer and L. M. Stephenson, *The Singlet Oxygen Ene Reaction*, *Singlet Oxygen, Vol. II*, *Reaction Modes and Products*, *Part 1*, ed. A. A. Frimer, CRC Press, Inc., Boca Raton, 1985, ch. 3, p. 67.
11 (*a*) P. de Mayo, *Molecular Rearrangements*, Interscience Publishers, New York, 1963, part 1; (*b*) P. de Mayo, *Molecular Rearrangements*, Interscience Publishers, New York, 1964, part 2.
12 A. J. Bloodworth and H. J. Eggelte, *Endoperoxides*, *Singlet Oxygen Vol. II*, *Reaction Modes and Products*, *Part 1*, ed. A. A. Frimer, CRC Press, Inc., Boca Raton, 1985, ch. 4, p. 93.
13 J. A. Steele and E. Mosettig; *J. Org. Chem.*, 1963, **28**, 571.
14 S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, 1982, **30**, 4181.
15 O. M. Teme Centurión, *Synthesis and Biological Activity of Castasterone Analogues*, PhD Thesis, Universidad de Buenos Aires, Argentina, 1997.
16 H. Schneider, U. Buchheit, N. Becker, G. Schmidt and U. Siehl, *J. Am. Chem. Soc.*, 1985, **107**, 7027.