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SYNTHESIS OF 6(α,β)-AMINOCHOLESTANOLS AS ERGOSTEROL BIOSYNTHESIS INHIBITORS

Pierre Beuchet, Laïla El kihel, Michel Dherbomez, Georges Charles and Yves Letourneux*

Laboratoire de Synthèse et Etude de Substances Naturelles à Activités Biologiques, Université de La Rochelle, avenue Marillac, 17042 la Rochelle CEDEX 01, France.

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Abstract : Δ^7-5-Desaturase catalyses one of the last steps in ergosterol biosynthesis in fungi. Moreover Δ^5-unsaturation is necessary for the sparking function. Synthesis of three pairs of C-6 epimeric cholestanol derivatives are described as potential growth inhibitors. Preliminary results suggest that 6β-aminocholestanol is a potent antifungal agent.

Introduction : In the past twenty years, invasive fungal infections have increased dramatically, especially in an expanding population of immunocompromised patients such as those afflicted with AIDS, receiving organ transplants or undergoing cancer chemotherapy. Nosocomial fungal infections, mainly invasive candidiasis, pulmonary aspergillosis and *Pneumocystis carinii* pneumonia are now important causes of morbidity and mortality. Most of the clinically useful antifungal agents inhibit the biosynthesis of ergosterol, the principal sterol of yeasts and fungi,[1] or interact directly with ergosterol in membranes.[2] However, current treatments are fungistatic rather than fungicidal and can cause severe liver damage (First Pass Effect). Among them, morpholine derivatives - containing an amine function which is protonated in biological media - are analogues of the carbocationic high energy intermediates[3] involved in the $\Delta^8 \rightarrow \Delta^7$ isomerase and thus, inhibit this enzyme.[4]

Our previous work has shown that 7-aminocholesterol[5] inhibits the same target ; this compound is fungicidal and its mechanism of growth inhibition is different than those of other sterol biosynthesis inhibitors already known. Another enzyme, Δ^7-5-desaturase, catalyses one of the last steps in ergosterol biosynthesis by removal of H-5α and H-6α during the desaturation process.[6]

Moreover, the Δ5-unsaturation plays a critical role for the sparking function.[7] Inhibitors structurally similar to the natural substrate 7,22-(E)-ergostadien-3β-ol with α-face oxygenated substituents[8] in the vicinity of carbon 5 exhibited weak potency (IC_{50}: 47-149 μM) against Δ^7-5-desaturase, but no antifungal activity was reported.[9] Our approach in this paper was to confirm that cholestanol should be a suitable framework for the elaboration of new ergosterol biosynthesis inhibitors. Thus, taking into account our

* Fax : (33) 05 46 45 82 27. e-mail : lelkihel@cri.univ-lr.fr

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previous results in aminosteroids fields, we performed the synthesis of the two epimeric 6α-amino (**1**) and 6β-aminocholestanol (**2**) (figure 1).

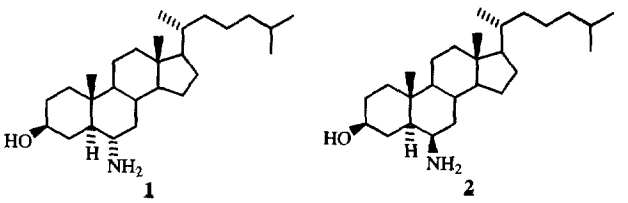


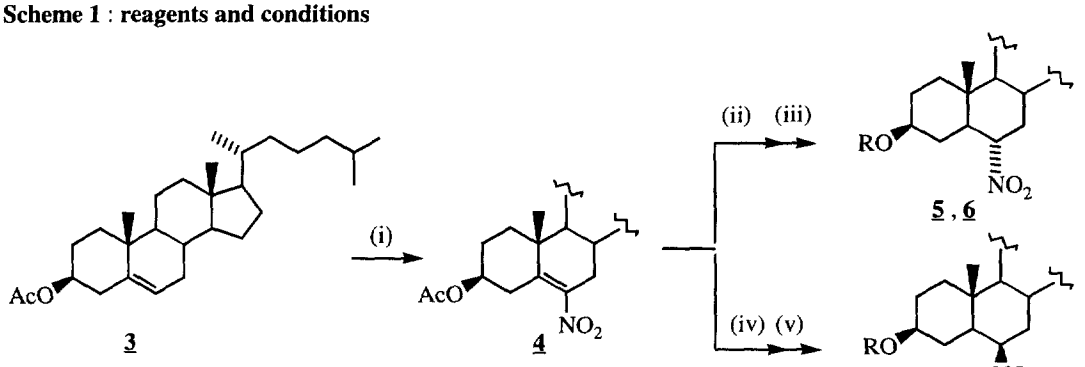
Figure 1

Chemistry : the general synthetic procedure used to prepare compounds **1** and **2** is shown in scheme 1. Cholesteryl acetate **3** first reacted with high density (d=1.53) nitric acid in anhydrous acetic acid[10] to give the 6-nitro derivative **4**.[11] The key step was reduction of the Δ5 double bond. According to Fetizon,[12] treatment of **4** with $NaBH_4$ in refluxing ethanol for 24 hours gave the 6α-NO_2 **5** derivative in high yield. If the same treatment was carried out for ten minutes at room temperature, the 6β-NO_2 **7** was synthesized.

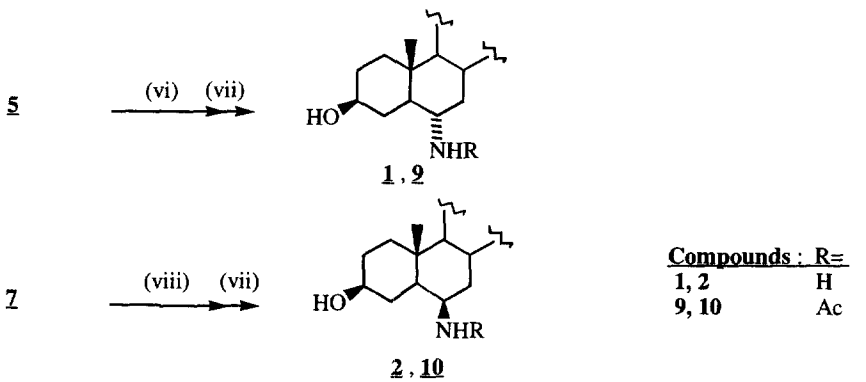
6α-Amino-5α-cholestan-3β-ol[13] **1** was obtained by nitro group reduction of 6α-nitro-5α-cholestanyl acetate **5** with aqueous hydrazine and Pd/C in ethanol, followed by saponification. However, in order to avoid epimerization[14] (of the nitro group) under basic conditions, both nitro and acetoxy groups of 6β-nitro-5α-cholestanyl acetate **7** were simultaneously reduced with $LiAlH_4$ in diethylether to yield compound **2**.[15] Compounds **6**[16] and **8**[17] were prepared by classical methods.

6-Acetamidosterols **9**[18] and **10**[19] were straightforwardly synthesized from the corresponding aminosterols **1** and **2** with acetic anhydride in methanol. All structures were unambiguously assigned on the basis of IR, ^1H and ^{13}C NMR, or MS spectral data.

Scheme 1 : reagents and conditions



Compounds :	R=
6, 8	H
5, 7	Ac



(i) acetic acid, HNO_3 (d=1.53, (80% ; **4**); (ii) $NaBH_4$, EtOH, reflux 24 h, (86% ; **5**) ; (iii) KOH 5%, EtOH 95%, (90% ; **6**) ; (iv) $NaBH_4$, EtOH, RT, 10 minutes, (88% ; **7**) ; (v) EtOH, hydrazine, 40-50°C, (20% ; **8**) ; (vi) aqueous hydrazine, Pd/C 10%, EtOH reflux, 48 h, (94% ; **1**) ; (vii) : Ac_2O, MeOH, 2 h, (88% ; **9**) ; (79% ; **10**) ; (viii) $LiAlH_4$, Et_2O reflux, 24 h (60% ; **2**).

Biological results and discussion : all compounds (**1**,**2**,**6**,**8**-**10**), containing a C6-nitrogen function, were tested[20] against *Saccharomyces cerevisiae* and *Candida albicans* (**table 1**). Antifungal activity was found to be related to the presence of the amino group ; 6-β-aminocholestanol **2** being four times more potent than the epimeric 6α-derivative **1**. These preliminary results confirm our previous studies that sterols containing an amino function in key positions interfering with the biosynthesis of ergosterol, are an interesting approach for antifungal compounds. Contrary to nitro **6**, **8** and acetamido **9**, **10** derivatives, 6-aminocholestanols **1**,**2** are protonated in biological media and we assumed that these compounds interfere as a mimic of the carbocationic high energy intermediate involved in the Δ^7-5-desaturase.

Table 1 : Growth Inhibition of *Saccharomyces cerevisiae* (ATCC 28 383) and *Candida albicans* (CIP 1180.79) by the 6R-(α,β)-substituted cholestanols.

Compound N° :	R	*S. cerevisae.**	*C. albicans**
1	α-NH_2	<15	<31
2	β-NH_2	<15	<7
6	α-NO_2	IN	IN
8	β-NO_2	IN	IN
9	α-NHAc	SD	SD
10	β-NHAc	SD	SD

*IC_{50} (Inhibitory Concentration) values are in μM. IN = Inactivity (over 250 μM). SD = Slow Development : 80 μM< IC_{50}<100 μM

Conclusion : most antifungal agents are hydrophilic and exhibit poor uptake in some tissues. Potentially lymphotropic antifungal drugs, such as 6-aminocholestanol derivatives, are promising candidates in combating systemic and deeply invasive infections. Further work is in progress to determine precisely the antifungal mechanisms.

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11. **3β-acetoxy-6-nitrocholest-5-ene** : ^{1}H NMR (400 MHz, $CDCl_3$) : 0.68 (s, 3H, Me-18) ; 0.91 (d, 3H, Me-21, J=6.4 Hz) ; 1.13 (s, 3H, Me-19) ; 2.1 (s, 3H, CH3-CO) ; 4.6-4.7 (m, 1H, H-3α). ^{13}C RMN ($CDCl_3$) : 11.7 (C-18) ; 18.6 (C-21) ; 19.7 (C-19) ; 20.9 ($\underline{CH_3}$-CO) ; 21.4(C-11) ; 71.9 (C-3) ; 137.8 (C-6) ; 146.6 (C-5) ; 170.1 (C=O). IR (KBr) ν (cm^{-1}) : 1739 (O-$\underline{C}$=O) ; 1529, 1366 (N=O). mp = 103-104°C. Litt[14] : 104-105°C. EI-MS, m/z (rel. intensity %) : 473 ($M^{+\cdot}$) ; 413 (44) ; 396 (72) ; 383 (60) ; 329 (25) ; 300 (100).
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13. **6α-amino-5α-cholestan-3β-ol** : ^{1}H NMR (400 MHz, $CDCl_3$ + CD_4O) δ : 0.64 (s, 3H, Me-18) ; 0.83 (s, 3H, Me-19) ; 0.89 (s, 3H, Me-21, J= 6.4 Hz) ; 2.94 (td, 1H, H-6β, J_t= 2 Hz, J_d=10.8 Hz) ; 3.53 (m, 1H, H-3α). ^{13}C NMR ($CDCl_3$ + CD_4O) δ : 11.3 (C-18) ; 12.2 (C-19) ; 17.9 (C-21) ; 20.5 (C-11) ; 50.0 (C-6) ; 69.3 (C-3). IR (KBr) ν (cm^{-1}) : 3600-3000 (OH and NH_2). EI-MS, m/z (rel. intensity %) : 403 ($M^{+\cdot}$) ; 386 (7) ; 273 (3) ; 255 (3) ; 248 (100) ; 155 (6).
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16. **6α-nitro-5α-cholestan-3β-ol** : ^{1}H NMR (400 MHz, $CDCl_3$) δ : 0.65(s, 3H, Me18) ; 0.86(d, 3H, Me27, J=6.4 Hz) ; 0.86(d, 3H, Me 26, J=6.4 Hz) ; 0.87 (s, 3H, Me 19) ; 0.90 (d, 3H, Me 21, J=6.6 Hz) ; 3.54-3.62 (m, 1H, H-3α) ; 4.41 (dt, 1H, H-6β, Jd= 2Hz, Jt=6.6 Hz). ^{13}C NMR ($CDCl_3$) δ : 11.9 (C18) ; 13.1 (C19) ; 18.6 (C21) ; 20.9 (C11) ; 70.1 (C3) ; 87.8 (C6). IR (KBr) ν(cm^{-1}) : 3600-3200 (OH) ; 1545, 1372(N=O). mp = 102°C.
17. **6β-nitro-5α-cholestan-3β-ol** : ^{1}H NMR (400 MHz, $CDCl_3$) δ : 0.73 (s, 3H, Me 18) ; 0.79 (s, 3H, Me 19) ; 0.86 (d, 3H, Me 27, J=6.8 Hz) ; 0.87 (d, 3H, Me 26, J=6.8 Hz) ; 0.91 (d, 3H, Me 21, J=6.4 Hz) ; 3.56-3.62 (m, 1H, H-3α) ; 4.38 (d, 1H, H-6α, J=2 Hz). ^{13}C NMR ($CDCl_3$) δ : 12.1 (C18) ; 13.2 (C19) ; 18.7 (C21) ; 20.1 (C11) ; 71.5 (C3) ; 84.4 (C6). IR (KBr) ν (cm^{-1}) : 3600-3200 (OH); 1547, 1370 (N=O). mp =102-104°C.
18. **6α-acetamido-5α-cholestan-3β-ol** : ^{1}H NMR (400 MHz, $CDCl_3$) δ : 0.64 (s, 3H, Me 18) ; 0.86 (d, 3H, Me 27, J=6.4 Hz) ; 0.86 (d, 3H, Me 26, J=6.4 Hz) ; 0.89 (d, 3H, Me 21, J= 6.4 Hz) ; 0.89 (s, 3H, Me 19) ; 1.96 (s, 3H, $\underline{CH_3}$-CO) ; 3.44-3.52 (m, 1H, H-3α) ; 3.80 (ddd, 1H, H-6β, J= 4Hz, J=8.8 Hz, J=12 Hz) ; 5.08 (d, 1H, NH, J= 9.6 Hz). ^{13}C NMR ($CDCl_3$) δ : 11.2 (C18) ; 13.3 (C19) ; 18.6 (C21) ; 21.1 ($\underline{CH_3}$-CO) ; 52.2 (C6) ; 71.4 (C3) ; 169.4 (C=O). IR (KBr) ν (cm^{-1}) : 3600-3200 (OH) ; 1660 (NH-$\underline{CO}$). mp = 256°C.
19. **6β-acetamido-5α-cholestan-3β-ol** : ^{1}H NMR (400 MHz, $CDCl_3$) δ : 0.69 (s, 3H, Me 18) ; 0.86 (d, 3H, Me 27, J=6.4 Hz) ; 0.86 (d, 3H, Me 26, J=6.4 Hz) ; 0.91 (s, 3H, Me 21, J= 6.4 Hz) ; 0.99 (s, 3H, Me 19) ; 1.98 (s, 3H, $\underline{CH_3}$-CO) ; 3.63-3.74 (m, 1H, H-3α) ; 4.12 (dd, 1H, H-6α, J= 6.8 Hz, J=13.2 Hz) ; 5.49 (d, 1H, NH, J= 11.2 Hz). IR (KBr) ν (cm^{-1}) : 3600-3200 (OH) ; 1665 (NH-$\underline{CO}$). mp = 250°C.
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