Synthesis of 14-α-Aminomethyl substituted Lanosterol Derivatives; Inhibitors of Fungal Ergosterol Biosynthesis

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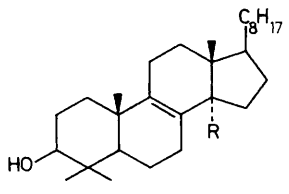
Several 14-α-aminomethyl-substituted lanosterol derivatives have been synthesized involving a complete $\Delta^{7,8}$ to $\Delta^{8,9}$ isomerization; these compounds are inhibitors of fungal ergosterol biosynthesis and are active against intact *Candida* and dermatophyte strains.

Ergosterol is an important constituent of the fungal cell membrane and has also been found in the mitochondria associated with mitochondrial enzymes.[1,2] An important step in the fungal biosynthesis of ergosterol is the cytochrome P_{450} mediated 14-demethylation of lanosterol (**I**). This P_{450} monooxygenase enzyme oxidatively removes the 14-methyl group by three O_2-NADPH dependent steps.[3] Inhibitors of this enzyme are not only potentially useful as cholesterol lowering agents[4] but also as antifungal agents. Studies have shown that the imidazole (Im) and triazole antifungals inhibit this enzyme by the binding of the heterocyclic nitrogen atom (N-3 of imidazole and N-4 of triazole) to the protoheme iron atom. This results in the exclusion of oxygen that would normally take part in the reaction.[5] It is further thought that the non-heterocyclic portion of the antifungal molecule binds to the lipophilic sites of cytochrome P_{450}.[5] Since the target of the enzyme is the 14-methyl position of lanosterol, a logical inhibitor could be lanosterol with a heme binding component (R = CH_2Im) at the 14-methyl position. We, therefore, set out to synthesize several 14-aminomethyl substituted lanost-8-ene-3-ols and specifically 14-1*H*-imidazolylmethyl lanost-8-ene-3-ol where R = CH_2-imidazole. An example of this approach is shown in the reported antifungal activity of the azasterol antibiotics,[6] and more recently, a series of 14-methyl-functionalized lanost-7-ene-3-ols were synthesized where R = CHF_2, CH_2CHF_2, CH_2CCH, $CH{=}CH_2$, and CCH. These compounds were shown to be inhibitors of the cytochrome P_{450}-14-demethylase enzyme.[7]

Typical methods of introducing a 1H-alkylimidazole group involve the nucleophilic displacement of alkyl halides or alkyl sulphonates with imidazole. Presumably a 14-methyltosylate or halide could be prepared from the known 14-hydroxymethyl lanosterol,[8] however displacement with a nucleophile such as imidazole adjacent to this neopentyl centre could prove extremely difficult. For this reason we introduced an amine at the 14-methyl position and built up the imidazole ring.

Our strategy (Scheme 1) utilized the photolysis of 3β-acetoxylanost-7α-nitrite (**1**) to obtain the oxime (**2**).[8] Attempted reduction of the oxime with either lithium aluminium hydride (LAH), LAH–$AlCl_3$, or Red-Al failed to give the desired 14-aminomethyl compound. However, hydrogenation using Pt catalysis with perchloric acid (1.1 equiv.) gave the desired compound in 25% yield. This low yield prompted us to investigate the reduction of the 14-nitrile (**3**) obtained by dehydration of (**2**) with Ac_2O–pyridine. LAH Reduction of (**3**) followed by aqueous workup gave the expected aldehyde.[8] Even under forced conditions of excess LAH and higher temperatures no aminomethyl compound was detected. However, treatment with 1:1 LAH–$AlCl_3$ gave the desired aminomethyl compound (**4**)† in 82% yield. Compound (**4**) was then dehydrated using HBr–HOAc to give a 4:1 mixture of alkenes (**5**) and (**6**), respectively as determined by the integration of the 7-alkenic proton at δ 5.23.



(**1**)

The (**5**,**6**) mixture was then treated with both strong acid and base under both protic and aprotic conditions with no apparent change in the ratio of (**5**) to (**6**). However, when a solution of the (**5**/**6**) mixture in methanol was adjusted to pH 4.5 with 1 M HCl we observed a complete isomerization of the 7,8-ene to give the desired 8,9-ene compound (**6**)† as determined by the complete disappearance of the 7-alkenic proton at δ 5.23. Complete isomerization apparently took place only in the presence of an amine group at the 14-methyl position. Thus when the 14-hydroxymethyl (**13**), or 14-nitrile (**10**) compounds (Scheme 2) with the double bond at the 7,8-position were treated at pH 4.5 under similar conditions, no isomerization was observed. When lanosterol is treated with strong acid, an equilibrium mixture of the $\Delta^{7,8}$ and $\Delta^{8,9}$ compounds is obtained.[9] Presumably, a similar equilibrium is set up with the 14-α-aminomethyl compound to give a 4:1 ratio of the $\Delta^{7,8}$ to $\Delta^{8,9}$ compounds under similar strongly acidic conditions. Therefore a plausible mechanism for this $\Delta^{7,8}$ to $\Delta^{8,9}$ isomerization may involve the intramolecular protonation of the 7,8-double bond by the proximal 14-ammonium ion at pH 4.5.

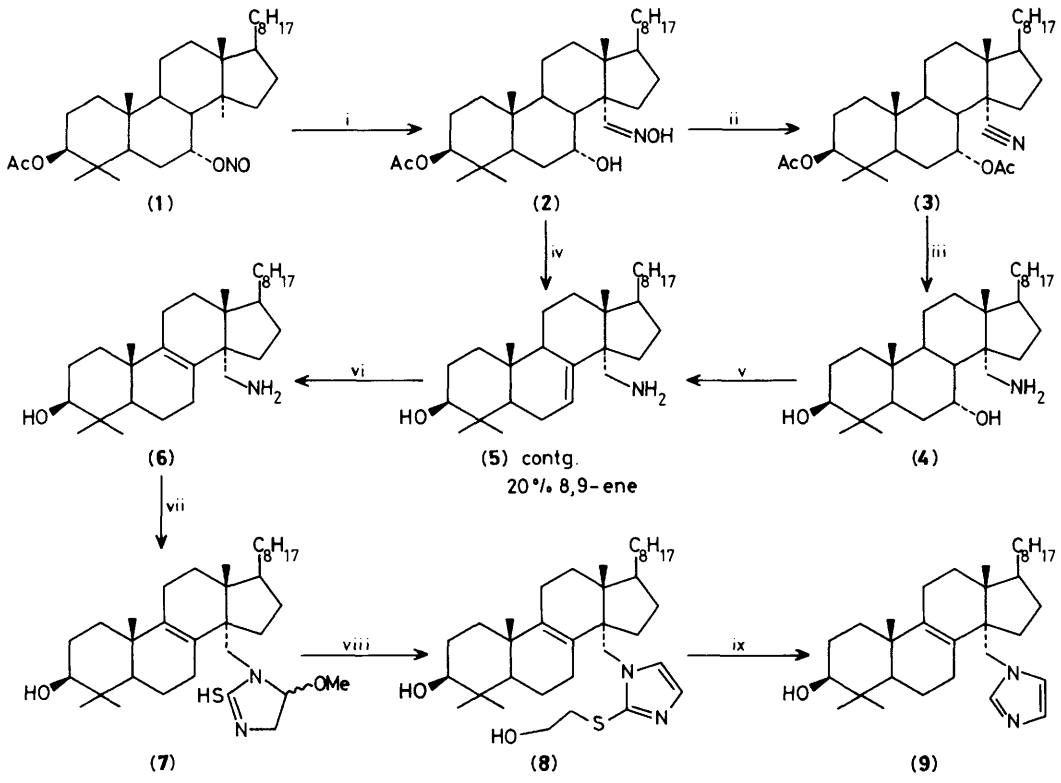
An alternative to the reduction of (**3**) to obtain (**6**) was to dehydrate (**2**) with MsCl–collidine to give the 7,8-ene-14-nitrile followed by LAH–$AlCl_3$ reduction to give (**5**), albeit in a lower yield than the previous route. Again, treatment of (**5**)

† All compounds gave elemental analyses consistent with structural assignments. All n.m.r. data obtained at 200 MHz in $CDCl_3$ unless otherwise noted. (**4**): m.p. 258—260 °C; ^{1}H n.m.r. δ 0.75—1.9 (envelope), 2.88 (2H, apparent s, 14-α-CH_2N), 3.33 (1H, dd, *J* 10 and 4 Hz, 3-α-H), 3.95 (1H, br.s, 7-β-H); electron impact (e.i.) m.s. *m/z* (rel. intensity) 462 (*M* + 1, 4), 461(*M*, 11), 364(41), 299(100).

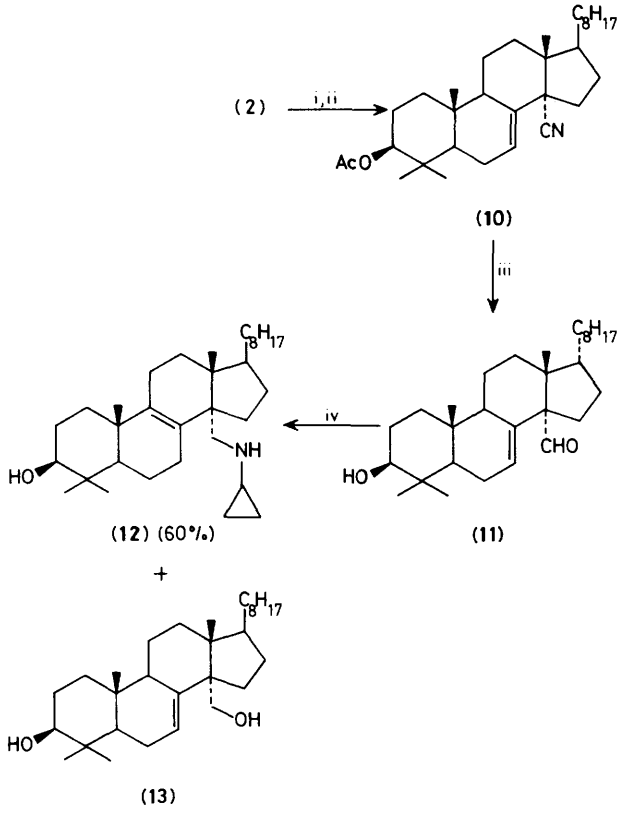
(**6**): m.p. 167—168 °C; ^{1}H n.m.r. [5% $(CD_3)_2SO$] δ 0.8—2.0 (envelope), 2.73 (2H, br.s, 14-α-CH_2N) superimposed on 2.5—2.8 (2 exchangeable H), 3.23 (1H, dd, *J* 10 and 4 Hz, 3-α-H); fast atom bombardment (f.a.b.) *m/z* (rel. intensity) 444(*M* + 1, 100), 427(54), 426(53), 409(57).

(**9**): ^{1}H n.m.r. δ 0.8—2.2 (envelope), 2.82—2.98 (1H, m), 3.31 (1H, dd, *J* 10 and 4 Hz, 3-α-H), 3.92 (2H, dd, *J* 8 and 4 Hz, 14-α-CH_2-Im), 6.89 (1H, s), 7.06 (1H, s), 7.45 (1H, s); e.i.m.s. *m/z* (rel. intensity) 494(*M*, 17), 413(25), 395(35), 135(27), 82(100).

(**12**): ^{1}H n.m.r. δ 0.22—0.5 (4H, m, cyclopropyl), 0.7—2.1 (envelope), 2.48 (2H, apparent br.s), 3.25 (1H, dd, *J* 10 and 4 Hz); e.i.m.s. *m/z* (rel. intensity) 483(*M*, 12), 414(15), 413(17), 412(23), 323(37), 225(44), 197(49), 70(100).



Scheme 1. *Reagents and conditions*: i, *hν*, Ph; ii, Ac_2O–Pyr (Pyr = pyridine), 93%; iii, LAH–$AlCl_3$, 82%; iv, MsCl–Pyr followed by refluxing in collidine, followed by LAH–$AlCl_3$, 32%; v, HBr–HOAc followed by NaOH, 83%; vi, pH 4.5/aq. MeOH, 100%; vii, $(MeO)_2CHCH_2NCS$, 75.8%; viii, $(CH_2OH)_2$/HCl, 92.5%; ix, Raney-Ni, 73%.



Scheme 2. *Reagents and conditions*: i, MsCl–Pyr; ii, collidine/reflux, 70%; iii, LAH, Et_2O, 42%; iv, cyclopropylamine, $NaCNBH_3$, pH 4.5.

Table 1. Biological activity of compounds (**6**) and (**9**).

Compound	MICs (mcg/ml, SDB) *Candida*	MICs (mcg/ml, SDB) Dermatophyte	Ergosterol inhibition IC_{50}/μM
(**6**)	11.9	7.6	0.3
(**9**)	26.3	9.2	0.03
(**12**)	>95.1	9.2	1.0
KTZ	>28.8	12.1	0.03

in MeOH at pH 4.5 resulted in complete isomerization to give (**6**) (Scheme 1).

The imidazole ring was then prepared by the reaction of (**6**) with dimethoxyethylisothiocyanate to give (**7**).[10] Acid hydrolysis in ethylene glycol gave (**8**), which was then desulphurized with Raney-Ni to give (**9**)† in 51% overall yield from the aminomethyl compound (**6**).

N-Alkylated cyclopropylamines have been reported to be suicide inhibitors of P_{450} mono-oxygenase enzymes.[11] Since the azole antibiotics are reversible inhibitors of cytochrome P_{450} we prepared the 14-cyclopropylaminomethyl compound (**12**). We utilized the known 14-aldehyde (**11**) which was obtained from the 7α-hydroxy-14-oxime (**2**) (Scheme 2).[8] Upon reductive amination of (**11**) with cyclopropylamine at pH 4.5 with $NaCNBH_3$ we obtained the cyclopropylaminomethyl compound (**12**)† in 60% yield (with the double bond again isomerized completely to the 8,9 position) plus the 14-hydroxymethyl compound (**13**) (double bond still in the 7,8 position). This was further proof that an amine was required at the 14-methyl position for complete 7,8 to 8,9 double bond isomerization.

The biological results (Table 1) show that compounds (**6**)

and (**9**) were more active in minimum inhibitory concentration (MIC) tests than ketoconazole (KTZ); Dermatophyte strains appeared to be more sensitive to these compounds than *Candida* strains.‡ All compounds tested were ergosterol biosynthesis inhibitors as measured by a decreased level in (2-^{14}C) mevalonic acid incorporation into ergosterol, and an accumulation of radioactive lanosterol in a cell free system.[12] It is noteworthy that the imidazole compound (**9**) was as effective an inhibitor as KTZ. Although all the compounds tested were inhibitors of ergosterol asynthesis, there was no direct correlation between IC_{50}s and MICs.

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12 The IC_{50} is the concentration of the compound resulting in 50% reduction of incorporation of α(14C)mevalonic acid into ergosterol. A cell-free extract of *C. albicans* was obtained by disruption of the cells by shaking with glass beads. Ergosterol synthesis was measured by the procedure described by Mitropoulos, *Biochim. Biophys. Res. Commun.*, 1976, **71**, 892. The non-saponifiable fraction containing sterols was fractionated by h.p.l.c. as described, I. J. Sud and D. S. Feingold, *Antimicrob. Agents Chemother.*, 1983, **23**, 185.

‡ MICs were performed in sabauroud dextrose broth and are expressed as the geometric mean. *Candida* strains: 4, *C. albicans*; 1, *C. tropicalis*; 1, *Trichophyton mentagrophytes, T. rubrum; Microsporum canis; M. gypseum, Epidermophtyon floccosum.*