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**Laboratory note**

# Synthesis and study of some 4-aza and 17a-azasteroidal isoxazoles

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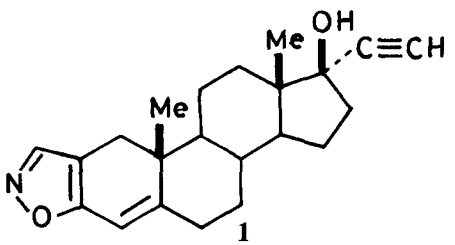
**Abstract** – 4-Aza-5 α-androstano[2,3-*d*]isoxazole **4** and 17a-aza-D-homo-5-androsteno[17,16-*c*]isoxazole **7** have been prepared by treating α,β-unsaturated-β-chloroaldehydes **3** and **6** [1, 2] with hydroxylamine hydrochloride in pyridine. [16,17-d]Isoxazole derivatives **4** and **8** were found to be unstable in most of the organic solvents. β-Cyanolactam derivatives **5** and **9** have also been prepared in both the series. © 1999 Éditions scientifiques et médicales Elsevier SAS

**azasteroids / isoxazoles / aromatase inhibitors / trilostane / danazol**

## 1. Introduction

It has already been shown that introduction of an isoxazole ring system in a steroidal moiety may lead to the formation of drugs of medicinal importance [3]. 17-α-Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-β-ol (**1**), danazol, an analogue of ethisterone, is a drug of interest for antifertility and other activities [4]. Danazol is used predominantly to suppress the pituitary and for treatment of hereditary angioneurotic oedema [5].

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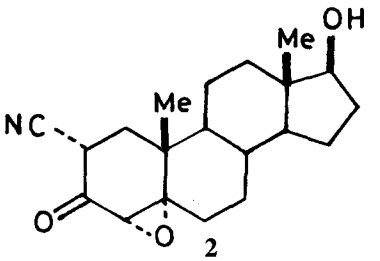
A cyanosteroid, trilostane, **2**, is an active inhibitor of steroid biosynthesis [6] and has been used in the treatment of Cushing's syndrome [7] when a more definite therapy cannot be utilized. It reduces adrenocortical synthesis of both cortisol and aldosterone and increases urinary excretion of 17-ketosteroids.

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These observations led us to synthesize isoxazole and cyano-bearing azasteroids, which we report in this communication.

## 2. Chemistry

For the preparation of 4-aza isoxazole (**4**), α,β-unsaturated-β-chloroaldehyde **3** [2] was treated with hydroxylamine hydrochloride in pyridine at room temperature. Interestingly, the product formed, **4**, was unstable in most of the organic solvents (eg. acetone and methanol) at boiling temperature, but upon crystallization from ether, **4** was obtained. UV maximum was observed at 292 nm, which shifted to 274 nm on alkali treatment [3]. $^1$H NMR singlet was observed at δ 8.16 (1H,3′-C*H*) and a triplet at δ 4.66 (1H, 17 α-*H*). The compound **4** on

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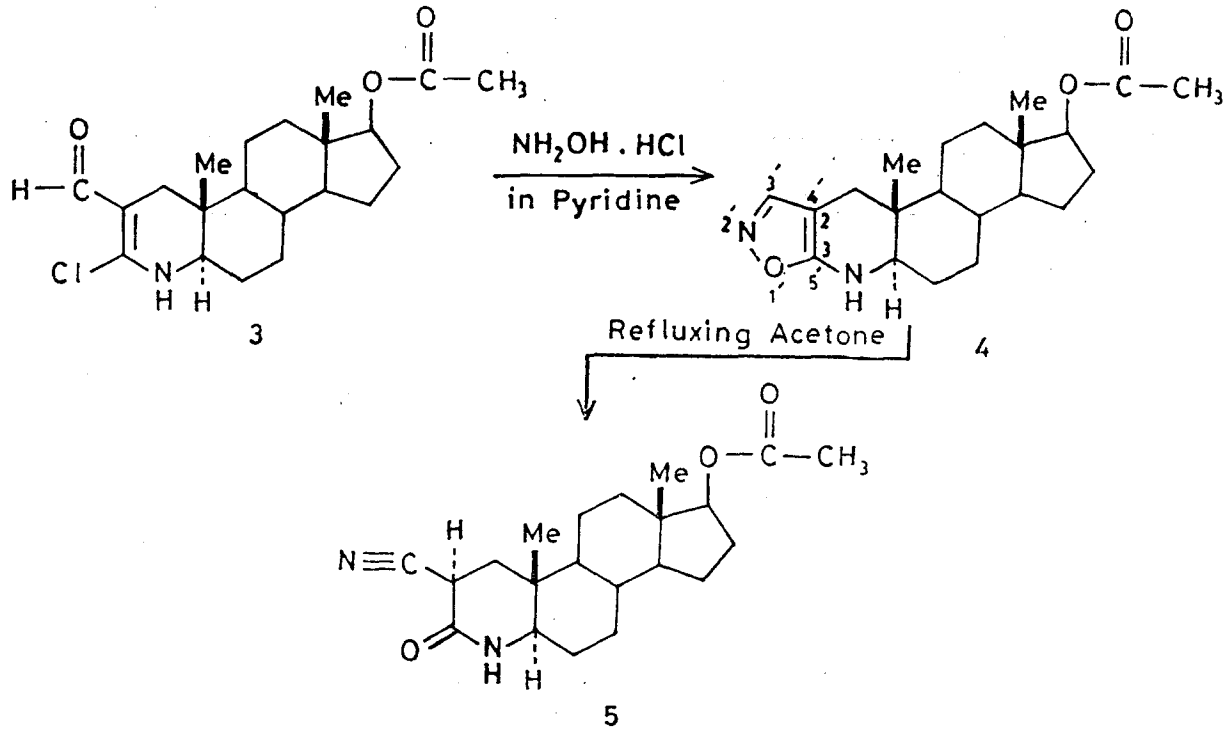



**Scheme 1.**

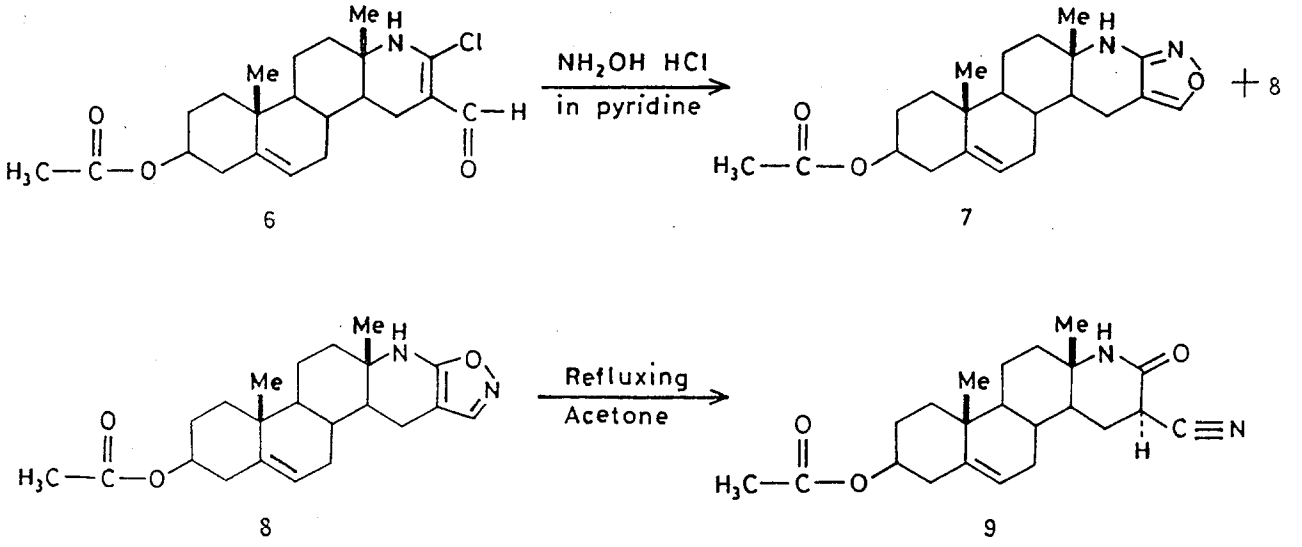
refluxing in acetone afforded β-cyanolactam **5**, which exhibited UV maximum at 219.2 nm and showed a bathochromic shift to 266.3 nm in alkaline methanol. In IR spectrum, vibrational bands appeared at 2 252 (C≡N) and 1 726 $cm^{-1}$ (-$OCOCH_3$). $^1H$ NMR spectrum showed a multiplet at δ 3.53 for 2α-*H*. The configuration at carbon-2 of **5** has been assigned on the basis of earlier reports [8]. Elemental analysis confirmed the structure of compound **5**.

In a similar way, 17-chloro-16-aldehyde **6** [1] was treated with hydroxylamine hydrochloride in pyridine at room temperature. After processing, a product was obtained which revealed two spots on TLC studies, indicating a mixture of [17,16-*c*] **7** and [16,17-*d*] **8** isoxazole derivatives. The mixture exhibited singlets at δ 7.98 (5′-C*H*) **7** and 8.23 (3′-C*H*) **8** in 30:70 area ratio, both together integrating for one proton. As discussed earlier, [16,17-*d*] isomer **8** in the mixture was also found to be unstable in the organic solvents at boiling temperature. The mixture of **7** and **8** was not separated and upon refluxing in acetone and further concentration to crystallization, afforded the cyanolactam derivative **9** [UV maximum at 219 nm, shifting to 266.2 nm in alkaline methanol, vibrational bands at 2 249 (C≡N), 1 735 (-$OCOCH_3$) and 1 674 $cm^{-1}$ (CONH), $^1H$ NMR singlet at δ 1.20 (18-C$H_3$) and multiplet at 3.56 (16 α-*H*)]. The configuration at carbon-16 in compound **9** is analogous to earlier observations [8]. The solid residue obtained from the mother liquor was treated with ether to afford crystallized [17,16-*c*]isoxazole derivative **7**. It showed UV maximum at 262 nm and no shift was observed on alkali treatment [3]. An infrared band appeared at 1 726 $cm^{-1}$ (-$OCOCH_3$). $^1H$ NMR singlets at δ 1.02 (18-C*H3*) and 7.98 (5′-C*H*). On the basis of spectral analyses, the compound [17,16-*c*] isoxazole and [16,17-*d*] isoxazole have been assigned the structures **7** and **8**, respectively.

## 3. Biological activity

### *3.1. Aromatase assay in placental microsomes*

The aromatase enzymes inhibitory activity of compounds **4**, **5** and **9** was carried out by Dr Martin G. Rowlands at the Institute of Cancer Research, Royal Cancer Hospital, Drug Development Section, CRC laboratories, UK. The activity was monitored by quantitating




**Scheme 2.**

the tritiated water released from radio-labelled androstenedione during aromatization of estrone [9]. The compounds did not display any significant inhibition of aromatase activity at 1, 5 and 20 μM concentrations. This suggested that the compounds did not have the ability to interact competitively with the substrate binding site or to get converted to reactive species to block the effect of estrone synthetase.

### *3.2. Antineoplastic activity*

The compounds **4** and **5** were also tested at NCI, Bethesda, MD, USA, in vitro, against a cell panel consisting of 60 lines. A 48 h continuous exposure protocol was used and a sulforhodamine B (SRB) protein assay was used to estimate the cell viability or growth. None of the compounds showed statistically significant antineoplastic activity for further study.

## 4. Experimental protocols

### *4.1. Chemistry*

Melting points reported are uncorrected. NMR spectra were taken with an AC–300 F Bruker spectrometer using $CDCl_3$ solutions with TMS as an internal standard. IR and UV spectra were obtained with Perkin Elmer 882 and Lambda–15 spectrophotometers, respectively. Mass spectra were obtained on a VG–11–250 J 70S model. Elemental analyses were carried out on a Perkin Elmer–2400 model. The purity of the compounds was examined by thin layer chromatography. All solvents were dried and freshly distilled prior to use.

#### *4.1.1. 4-Aza-5-α-androstano[2,3-d]isoxazol-17-β-yl acetate* **4**

To a stirred solution of hydroxylamine hydrochloride (0.16 g) in dry pyridine (3 mL) at room temperature was added 17-β-acetoxy-3-chloro-4-aza-5-α-androst-2-en-2-aldehyde **3** (0.1 g) under anhydrous conditions. The reaction mixture was stirred for 45 min and poured into ice cold water. The precipitate obtained was filtered, washed thoroughly with water, dried and crystallized from freshly distilled solvent ether to afford **4**. Yield: 0.08 g (85%); m.p.: 272–278 °C (decomp.); $\lambda_{max}$ MeOH 292 nm (log ε 4.06), $\lambda_{max}$ (0.1 N KOH–MeOH) 274 nm; IR (KBr): 3 284 (-NH), 1 712 (-$OCOCH_3$); NMR ($CDCl_3$): δ 0.80 (6H, s, 18-C*H*$_3$ and 19-C*H*$_3$), 2.04 (3H, s, -$OCOCH_3$), 2.96 (1H, m, 5-α-*H*), 3.84 (1H, s, exchangeable, -N*H*), 4.66 (1H, t, 17-α-*H*), 8.16 (1H, s, 3′-C*H*). Anal for $C_{21}H_{29}N_2O_3$: C, 70.50; H, 8.17; N, 7.84. Found: C, 70.12; H, 7.93; N, 7.43.

#### *4.1.2. 17-β-Acetoxy-2-β-cyano-4-aza-5-α-androstan-3-one* **5**

A solution of **4** (0.1 g) in acetone (30 mL) was refluxed for 30 min. The solution was concentrated and crystallized to afford **5**. Yield: 0.075 g (75.5%); m.p.: 288–290 °C; $\lambda_{max}$ MeOH 219.2 nm (log ε 3.17), $\lambda_{max}$ (0.1 N KOH–MeOH) 266.3 (log ε 3.85); IR (KBr): 3 201

(-NH), 2 252 (C≡N), 1 726 (-$OCOCH_3$), 1 684 (lactam); NMR ($CDCl_3$): δ 0.70 (3H, s, 18-C*H*$_3$), 0.86 (3H, s, 19-C*H*$_3$) 1.96 (3H, s, -OCOC*H*$_3$), 3.16 (1H, dd, 5 α-*H*), 3.53 (1H, m, 2-α-*H*), 4.50 (1H, d, 17-α-*H*), 6.50 (1H, s, exchangeable, -N*H*); MS: m/z 358. Anal for $C_{21}H_{29}N_2O_3$: C, 70.50; H, 8.18; N, 7.84. Found: C,70.20; H, 8.02; N, 7.64.

*4.1.3. 17a-Aza-D-homo-5-antrosteno[17,16-c] / [16,17-d]isoxazol-3-β-yl acetates* **7** *and* **8**

To a stirred solution of hydroxylamine hydrochloride (0.16 g) in dry pyridine (3 mL) was added 3-β-acetoxy-17-chloro-17a-aza-D-homo-5,16-androstadien-16-aldehyde **6** (0.1 g) under anhydrous conditions. The reaction mixture was stirred at room temperature for 20 min and poured into ice cold water. The precipitate obtained was filtered, washed, dried and crystallized from freshly distilled solvent ether to afford a mixture of **7** and **8**. Yield: 0.07 g (74.82%); m.p.: 198–200 °C (decomp.); $\lambda_{max}$ MeOH: 291 nm (log ε 3.13), $\lambda_{max}$ (0.1 N KOH–MeOH): 266.3 nm; IR (KBr): 3 347 (-NH), 1 732 (-$OCOCH_3$); NMR ($CDCl_3$): δ 1.00 (3H, s, 19 -C*H*$_3$), 1.10 (3H, s, 18 -C*H*$_3$), 2.03 (3H, s, -OCOC*H*$_3$), 4.10 (1H, s, exchangeable, -N*H*) 4.50 (1H, m, 3-α-*H*), 5.40 (1H, m, 6-C*H*), 7.98 (s) 8.23 (s) (30:70, 1H, 5′,3′-C*H*). Anal for $C_{22}H_{30}N_2O_3$: C, 71.32; H, 8.16; N, 7.56. Found: C, 71.10; H, 7.97; N, 7.24.

*4.1.4. 16-β-Cyano-17-oxo-17a-aza-D-homo-5-androsten-3-β-yl acetate* **9**

A solution of a mixture of **7** and **8** (0.1 g) in acetone (30 mL) was refluxed for 30 min. The solution was concentrated and allowed to crystallize to obtain **9**. Yield: 0.05 g (50%); m.p.: 298–300 °C (decomp.); $\lambda_{max}$ MeOH: 219 (log ε 2.87), $\lambda_{max}$ 0.1 N KOH–MeOH: 266.2 nm (log ε 4.20); IR (KBr): 3 183 (NH), 2 249 (C≡N), 1 735 (-$OCOCH_3$), 1 674 (lactam); NMR ($CDCl_3$-DMSO-$d_6$): δ 1.00 (3H, s, 19-C*H*$_3$), 1.20 (3H, s, 18-C*H*$_3$), 2.03 (3H, s, -OCOC*H*$_3$), 2.67 (1H, s, -N*H*), 3.56 (1H, m, 16-α-*H*), 4.60 (1H, m, 3-α-*H*), 5.43 (1H, m, 6-C*H*). Anal for $C_{22}H_{30}N_2O_3$: C, 71.31; H, 8.16; N, 7.56. Found: C, 70.92; H, 7.84; N, 7.46.

The mother liquor of **9** was dried and the residue was shaken with solvent ether. The insoluble cyano derivative was removed by filtration. The filtrate was concentrated and crystallized to give 17a-aza-D-homo-5-androsteno[17,16-*c*]isoxazol-3-β-yl acetate **7**. Yield: 0.025 g (25%); m.p.: 176–182 °C; $\lambda_{max}$ MeOH: 262 nm (log ε 3.70); IR (KBr): 3 354 (-NH), 1 726 (-$OCOCH_3$); NMR ($CDCl_3$): δ 1.00 (3H, s, 19-C*H*$_3$), 1.03 (3H, s, 18-C*H*$_3$), 2.03 (3H, s, -OCOC*H*$_3$), 4.40 (1H, s, exchangeable, -N*H*), 4.60 (1H, m, 3-α-*H*), 5.43 (1H, m, 6, -C*H*), 7.98 (1H, s, 5′ -C*H*); MS: m/z 370. Anal for $C_{22}H_{30}N_2O_3$: C, 71.31; H, 8.16; N, 7.56. Found: C, 71.12; H, 7.87; N, 7.46.

## Acknowledgements

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## References

[1] Jindal D.P., Pathak D., Eur. J. Med. Chem. 26 (1991) 651–654.

[2] Gupta R., Pathak D., Jindal D.P., Eur. J. Med. Chem. 31 (1996) 241–247.

[3] Manson A.J., Stonner F.W., Neumann H.C., Christiansen R.G., Clarke R.L., Ackerman J.H. et al., J. Med. Chem. 6 (1963) 1–9.

[4] Jenkin G., Aust N.Z., J. Obstet. Gynaecol. 20 (1980) 113–118.

[5] Zurlo J.J., Frank M.M., Fertil. Steril. 54 (1990) 64–72.

[6] Christiansen R.G., Neumann H.C., Salvador U.J., Bell M.R., Schane H.P. Jr., Creange J.E., Potts G.O., Anzalone A.J., J. Med. Chem. 27 (1984) 928–931.

[7] Komanicky P., Spark R.F., Melky J.C., J. Clin. Endocrinol. Metab. 47 (1978) 1042–1051.

[8] Mori Y., Tsuboi M., Suzuki M., Chem. Pharm. Bull. 29 (9) (1981) 2478–2484.

[9] Thompson E.A. Jr., Siiteri P.K., J. Biol. Chem. 249 (1974) 5364–5372.