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Synthesis and Anti-inflammatory Activity of Steroidal Isoxazoles

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The steroidal isoxazoles, 4-aza-5α-cholestano [3,2-c] isoxazole (2), 4-aza-5α-androstano [3,2-c] isoxazol-17β-yl acetate (4) and 17a-aza-D-homo-5-androsteno [17,16-c] isoxazol-3β-yl acetate (6) have been prepared and screened for their biological activity. The Compound 2 (ED_{50} = 5.67 mg/kg), Compound 4 (ED_{50} = 2.97 mg/kg) and Compound 6 (ED_{50} = 11.00 mg/kg) were found to be potent anti-inflammatory agents.

INTRODUCTION

There continues interest in the heteromodification of steroids. Heteromodification of glucocorticoids has led to the synthesis of several extremely potent anti-inflammatory agents[1–4]. Earlier, it was observed that all the steroidal compounds possessing anti-inflammatory activity have C-3 oxygen function. An attempt was made to synthesise anti-inflammatory agents lacking C-3 oxygen function. In the earlier studies, it was observed that certain pyrazole derivatives possess anti-inflammatory activity where oxygen at C-3 has been replaced with N.[5–6] Heterosteroids of medicinal interest have been reported from our research laboratory.[7–10] Therefore, it was considered worth while to fuse isoxazole moiety to the ring A of the steroid in cholestane series and ring A and D in the androstane series and to screen for their biological activities.

The initial work was carried out in cholestane series. 3-chloro-4-aza-5α-cholest-2-en-2-aldehyde (1) was prepared by the known procedure.[11] It showed a proton singlet at δ 9.80 in the NMR spectrum and showed a UV maximum at 302 nm. Refluxing 1 with hydroxylamine hydrochloride in the presence of potassium carbonate in aldehyde-free ethanol gave the product 2. The NMR spectrum showed a signal at 8.00 ppm for the hydrogen on the heterocyclic ring, *i.e.*, isoxazole ring and showed a UV maximum at 262 nm.

Similar sequence of reactions was carried out for the synthesis of 4-aza-5α-androstano [3,2-c] isoxazol-17β-yl-acetate (4). Testosterone acetate was oxidized with permanganate/periodate solution to give the seco-keto acid[12]. The acetoxy function was hydrolysed to hydroxy group under these reaction conditions. The seco-keto acid was subjected to the Leukart reaction resulting in the formation of lactam, then acetate was prepared, which on treatment with Vilsmeier-Haack reagent yielded 17β-acetoxy-3-chloro-4-aza-5β-androst-2-en-aldehyde (3).[11]

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Refluxing compound 3 with hydroxylamine hydrochloride in the presence of potassium carbonate in aldehyde-free ethanol gave the compound 4. The NMR spectrum showed a signal at 7.87 ppm for the hydrogen on the isoxazole ring and it also showed a UV maximum at 262 nm.

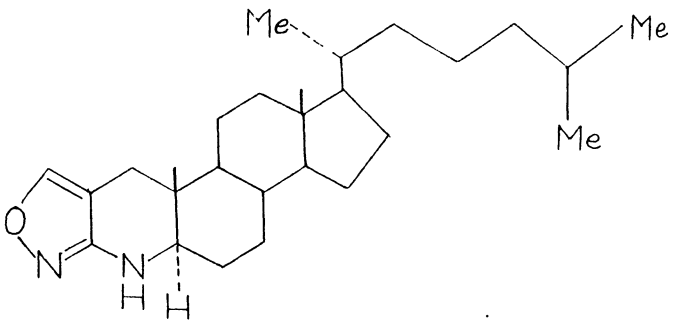
The synthesis of 17a-aza-D-homo-5-androsteno [17,16-c] isoxazol-3β-yl acetate (6) was then carried out. 3β-acetoxy-17-chloro-17a-aza-D-homo-5,16-androstadien-16-aldehyde (5) was prepared as reported earlier[5]. Treatment of compound 5 with hydroxylamine hydrochloride under similar conditions yielded the compound 6. The NMR spectrum of compound 6 showed a signal at 8.03 ppm for the hydrogen on the isoxazole ring attached to the D ring of steroidal nucleus and also showed a UV maximum at 265 nm.

The compounds 2, 4 and 6 were stable to alkali. Either of the compounds did not show opening of the isoxazole system on treatment with sodium methoxide which showed that the [3,2-c] system was present instead of [2,3-d] isoxazole in the compounds 2 and 4 and [17,16-c] isoxazole in the compound 6 instead of [16,17-d] isoxazole.

EXPERIMENTAL

The melting points reported are uncorrected. NMR spectra were recorded on a EM-390, 90 MHz model NMR instrument using tetramethylsilane (TMS) as the internal standard. IR spectra were obtained on Perkin-Elmer 137 spectrophotometer in a KBr pellet. Mass spectra were recorded on a Vg micro mass 7070F mass spectrometer. The structures of the compounds were established on the basis of their elemental analysis and spectral data.

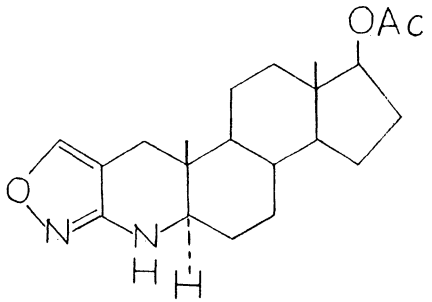
4-Aza-5α-cholestano [3,2-*c*] *isoxazole* (2): Potassium carbonate (0.1 g) was added to a refluxing solution of 3-chloro-4-aza-5α-cholest-2-en-2-aldehyde (1) (0.2 g) in aldehyde-free ethanol (50 mL). After 10 minutes hydroxylamine hydrochloride (0.1 g) was added and refluxed for 2 h. The reaction mixture was concentrated to 10 mL and poured into ice-cold water (100 mL). The precipitate thus obtained was washed thoroughly with water, dried and crystallised from



4-Aza-5α-Cholestano [3,2-c] isoxazole (2)

acetone to give 2. m.p. 136°C, yield 54.6%. Analysis: found (%) C 78.49, H 11.07, N 6.75, required C 78.58, H 10.75, N 6.80. UV max (MeOH): 262 nm. IR (KBr): 3175 cm^{-1} (N—H stretch), 2880 cm^{-1} (C—H stretch). NMR ($CDCl_3$): δ 0.70 (s, 3H), 0.93 (s, 3H), 2.97 (m, 1H), 4.13 (1H, disappeared on D_2O exchange) and 8.00 ppm (s, 1H). EIMS: m/z 412 (M^+).

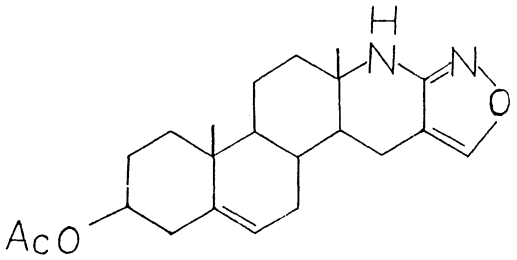
4-Aza-5α-androstano [3,2-c] isoxazol-17β-yl-acetate (4): Potassium carbonate (0.1 g) was added to a refluxing solution of 17β-acetoxy-3-chloro-4-aza-5α-androst-2-en-2-aldehyde (3) (0.1 g) in aldehyde-free ethanol (50 mL). Hydroxylamine hydrochloride (0.1 g) was added to the refluxing solution and further refluxed for 1 h. The reaction mixture was concentrated and then poured into ice-cold water (100 mL). The precipitate obtained was filtered, washed thoroughly with water, dried and crystallised from methanol to gave the compound 4 m.p. 185°C. Yield 42.4%. Analysis: found (%) C 70.34, H 8.35, N 7.55; required C 70.36, H 8.43, N 7.82. UV max (MeOH): 262 nm. IR (KBr): 3189 (N—H stretch), 1734 cm^{-1} (ester). NMR ($CDCl_3$): δ 0.83 (s, 3H), 1.26 (s, 3H), 2.03 (s, 3H), 4.50 (m, 1H) and 7.87 ppm (s, 1H). EIMS m/z 357 (M^+).



4-Aza-5α-androstano [3,2-c] isoxazol-17β-yl-acetate (4)

17a-Aza-D-homo-5-androsteno [17,16-c] isoxazol-3β-yl acetate (6): Potassium carbonate (0.3 g) was added to a refluxing solution of 17-chloro-16-formyl-17a-aza-D-homo-5,16-androstadien-3β-yl acetate (5) (0.3 g) in aldehyde-free ethanol (50 mL). Hydroxylamine hydrochloride (0.1 g) was added to the refluxing solution and was further refluxed for 1 h. The reaction mixture was concentrated and poured into ice-cold water. The precipitate obtained was filtered, washed thoroughly with water, dried and crystallized from methanol to give 6. m.p. 254°C. Yield 68.2%. Analysis: found (%) C 70.80, H 8.24, N 7.33; required C 71.33, H 8.16, N 7.56. UV max (MeOH): 265 nm, IR (KBr): 3125 cm^{-1} (N—H stretch), 2840 cm^{-1} (C—H stretch), 1730 cm^{-1} (ester). NMR ($CDCl_3$): δ 1.06 (s, 3H), 1.13 (s, 3H), 2.11 (s, 3H), 4.68 (m, 2H, 1H disappeared on D_2O exchange), 5.50 (m, 1H) and 8.03 ppm (s, 1H): EIMS: m/z 370 (M^+).

Anti-inflammatory activity: The screening was carried out using the corrageenan rat paw oedema model[13]. The compounds were given orally as suspensions. The extent of inhibition of oedema was related to the dose



7a-Aza-D-homo-5-androsteno [17,16-c] isoxazol-3β-yl acetate (6)

administered. For comparison, hydrocortisone was used as standard. Compounds 1, 3 and 5 which do not possess heterocycle fused to the azasteroid skeleton, were inactive in the dose range of 4–16 mg/kg. Whereas steroidal isoxazole 2 (ED_{50} = 5.67 mg/kg), 4 (ED_{50} = 2.97 mg/kg) and 6 (ED_{50} = 11.00 mg/kg) showed significant anti-inflammatory activity and were found to be more active than hydrocortisone (ED_{50} = 13.49) as shown in Table-1.

TABLE-1
ANTI-INFLAMMATORY ACTIVITY OF VARIOUS COMPOUNDS AND HYDRO CORTISONE ON CARRAGEENAN-INDUCED PAW OEDEMA IN RATS (OBSERVED 180 MINUTES AFTER THE ADMINISTRATION OF CARRAGEENAN)

Compound	Dose (mg/kg)	No. of rats	Inhibition of oedema (%) ± SEM	ED 50 (mg/kg)
1.	4.00	6	5.36 ± 1.44	
	8.00	6	6.50 ± 1.20	
	16.00	6	12.00 ± 1.44	ND
2.	4.00	6	45.28 ± 2.32	
	8.00	6	55.36 ± 1.28	
	16.00	6	61.48 ± 1.68	5.67
3.	4.00	6	12.36 ± 0.96	
	8.00	6	16.00 ± 7.39	
	16.00	6	21.51 ± 2.12	ND
4.	4.00	6	52.00 ± 1.44	
	8.00	6	58.64 ± 2.48	
	16.00	6	62.64 ± 1.60	2.97
5.	4.00	6	9.28 ± 2.28	
	8.00	6	12.44 ± 1.28	
	16.00	6	20.42 ± 1.46	ND
6.	4.00	6	21.40 ± 1.47	
	8.00	6	42.00 ± 1.92	
	16.00	6	55.06 ± 2.02	11.00
Hydrocortisone	4.00	6	18.80 ± 1.65	
	8.00	6	43.07 ± 4.75	
	16.00	6	59.24 ± 5.84	13.49

Significance relative to control group data $P < 0.01$, ND: Not determinable.

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