

Anti-Inflammatory and Anti-Diarrhoeal Activities of a Steroidal IndoxylF. N. KAMAU^{1*}, I. O. KIBWAGE¹, A. N. GUANTAI², G. MURIUKI² AND R. MUNENGE³¹*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, College of Health Sciences, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya.*²*Department of Pharmacology and Pharmacognosy, Faculty of Pharmacy, College of Health Sciences, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya.*³*Department of Veterinary Public Health, Pharmacology and Toxicology, College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 30197 Nairobi, Kenya.*

The anti-inflammatory and antidiarrhoeal activities of 3 β -Hydroxy-16, 17-seco-16-nor-5-androsten-15-(2-indoxyliden)-17-oic acid (I) are reported. After intraperitoneal administration, compound (I) gave an ED₅₀ of 9.5 mg/kg using the carrageenan induced rat paw oedema anti-inflammatory assay method. Indomethacin had an ED₅₀ of 5.8 mg/kg in this assay. Compound (I) and indomethacin caused comparable and dose-dependent varying degrees of delay in diarrhoea and also significantly reduced net colonic water flux into the colon of rats induced by castor oil.

Key words: Steroidal Indoxyl, Anti-Inflammatory, Antidiarrhoeal.

INTRODUCTION

Glucocorticoids and non-steroidal anti-inflammatory drugs (NSAID) constitute the major classes of anti-inflammatory agents in current use. Most of the acidic NSAID usually have in their structural features two hydrophobic moieties, often aromatic, one of which bears a carboxylic acid function [1]. The steroidal indoxyl (I) was chosen as a suitable candidate for anti-inflammatory screening since it resembles in its structural features, both corticosteroids and NSAID. NSAID also cause a significant delay in intestinal evacuations induced by castor oil challenge [2]. Ricinoleic acid, the active constituent of castor oil also reduces or reverses net water flux from the lumen to blood in the rat colon *in situ*, and pretreatment of rats with indomethacin significantly reduces this effect of ricinoleic acid on net water flux [3]. These effects have therefore been suggested as suitable methods for evaluation of prostaglandin biosynthesis inhibitors [2,3]. Ricinoleic acid causes fluid accumulation in the lumen of the intestinal tract, as well as damage to mucosa cell layers [4]. This inflammatory activity has led to parallels being drawn between the intestinal effects of ricinoleic acid and those of prostaglandins [5]. Compound I was synthesized as reported previously [6] by a

base catalyzed condensation between 3 β -hydroxy-5-androsten-17-one with 2-nitrobenzaldehyde, and its structure confirmed by spectroscopic and other methods.

MATERIALS AND METHODS

3 β -Hydroxy-5-androsten-17-one, indomethacin and carrageenan type IV were obtained from Sigma Chemical Company (St. Louis, MI, USA). 2-nitrobenzaldehyde was obtained from Aldrich Chemical Company (Gillingham, Dorset, England). Aspirin B.P. was obtained from Dawa Pharmaceuticals (Nairobi, Kenya). The rest of the reagents were laboratory reagent grade (BDH, Poole, U.K.). Melting point was determined with a Gallenkamp melting point apparatus (London, U.K.)

ANTI-INFLAMMATORY ACTIVITY

The carrageenan-rat paw oedema method was used [7]. Indomethacin, and compound I were suspended in 2% carboxymethylcellulose. Carrageenan 1% was prepared in normal saline. The test substances were administered intraperitoneally in dose volumes of 0.05 ml into male albino rats (180 \pm 20 g) one hour before

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injection of 0.1 ml carrageenan. Carrageenan was injected into the subplantar area of the left hind paw. The doses employed were 10.00, 5.00 and 3.00 mg/kg of indomethacin and 50.00, 25.00, 5.00 mg/kg of compound I. Six rats were used per dose. Control animals [6] received 0.5 ml of the vehicle.

The initial volume (V_i) was measured using the mercury displacement method. Three hours after carrageenan injection the final paw volume (V_f) was measured. The change in volume for controls (DV_c) and test (DV_t) was calculated as follows:

$$\begin{aligned} DV_c &= (V_f - V_i) \\ DV_t &= V_f - V_i \end{aligned}$$

The anti-inflammatory effect was evaluated from the % inhibition of oedema swelling relative to that in untreated control group. The % inhibition of oedema was calculated from the equation.

$$\% \text{ Oedema inhibition} = 100 \left(1 - \frac{DV_t}{DV_c} \right)$$

ANTI-DIARRHOEAL ACTIVITY

Preparation of test compounds

A 10 % castor oil emulsion in Tyrode solution was prepared using Tween 80 as the surfactant. This non-ionic emulgent was chosen to avoid the

irritant effects of ionic surfactants in the colon. Aspirin, indomethacin and the test compound I were dissolved or suspended in a vehicle containing 0.2% Tween 80 and 4% polythene glycol (PEG) 400 in distilled water.

Effect of aspirin, indomethacin and test compounds on castor oil induced diarrhoea in the rat

The method used was similar to that reported elsewhere [2]. Albino rats of both sexes, 150 ± 15 g, were starved overnight and treated orally by gavage the next morning with a selected dose of the drugs or test compound (4 and 10 mg/kg, 1 ml/100g). One hour later, 1 ml of the castor oil emulsion was administered orally, also by gavage. After castor oil challenge 1, 1½, 2, 2½, and 3 hour transparent plastic dishes placed beneath individual cages housing the rats were inspected for the presence of characteristic diarrhoea droppings; their absence was recorded as a positive result at that time, indicating protection from diarrhoea. Ten rats per drug or compound dose were used, and control rats (50) received the vehicle.

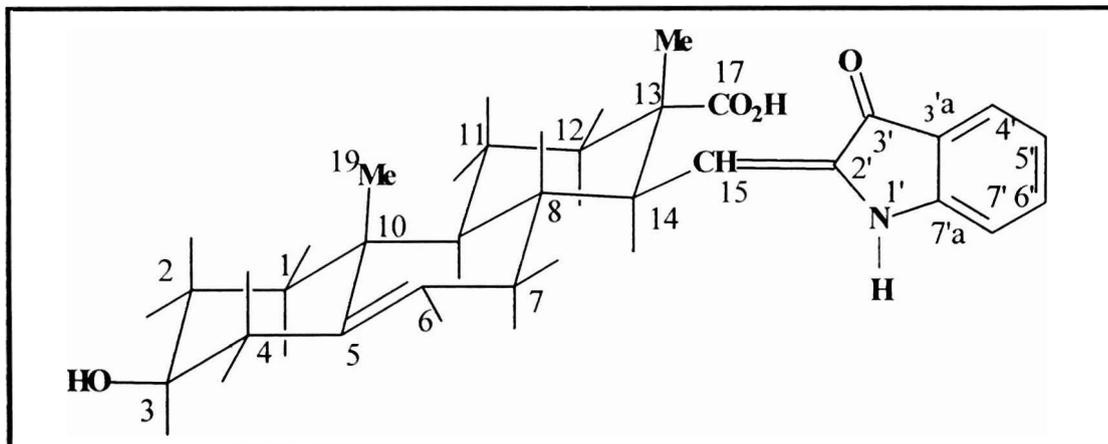


Figure 1: Structure of Compound I

Effects of drugs and test compounds on castor oil induced colonic water flux

A method similar to one reported [3] was used. Albino rats of both sexes, 150 ± 20 g, were starved for 24 h before the experiment. They were anaesthetized with urethane, 1.25 g/kg given intraperitoneally, and dissected to expose the colon. The entire colon was rinsed with 20 ml of warm saline solution (0.15 M NaCl) *in situ* by means of a syringe. 30 min later, the colon was filled with 2 ml of Tyrode solution and ligated. After 60 min, the colon was removed, weighed, opened at one end, emptied and reweighed. Net water flux was calculated from the weight of filled colon minus weight of empty colon minus 2 g initial instillate. A negative value denoted net water absorption from, and a positive value net water secretion into, the colon.

The entire experiment was repeated with: (a) 2 ml of castor oil emulsion instead of Tyrode solution (control). (b) 2 ml of castor oil emulsion instead of Tyrode solution, on rats pretreated with the test compound (4 mg/kg per day) intraperitoneally, for 2 days before the experiment. Six rats were used for each experiment.

RESULTS AND DISCUSSION

Anti-Inflammatory Activity

The ED₅₀ values for compound I and indomethacin were obtained from a plot of percentage inhibition of carrageenan rat paw oedema against log dose and were 9.5 and 5.8 mg/kg respectively. The log dose response curves for indomethacin and compound I were not parallel. It is discernible that the ED₅₀ for indomethacin compares with values reported elsewhere [2].

Anti-Diarrhoeal Activity

Table 1 shows the protection from castor oil diarrhoea afforded by various test compounds. The results show that the protection afforded by all test compounds at the earliest inspection time (1 h), and the duration of protection beyond 1 h, is dose dependent. At the lower dose of 4 mg/kg, indomethacin and compound I give complete protection at the 1 h inspection period.

Table 1: The percentage of rats protected from castor oil induced diarrhoea

Treatment	Dose mg/kg	Time (h)				
		1	1½	2	2½	3
Vehicle	-	0	-	-	-	-
Aspirin	4	10	0	-	-	-
Indomethacin	4	100	90	70	50	0
Compound (I)	4	100	80	50	20	0
Aspirin	10	90	40	0	-	-
Indomethacin	10	100	100	80	70	50
Compound (I)	10	100	100	100	70	20

At the higher dose level of 10 mg/kg aspirin does not give complete protection, at the 1 h inspection time. Indomethacin gives slightly better protection over the entire inspection period at both dose levels than compound I. This dose-dependent pattern of protection against castor oil diarrhoea has been reported previously for non-steroidal anti-inflammatory drugs. It was also reported then, that the relative potencies of some 31 of these drugs in the castor oil challenge and carrageenan foot oedema correlated significantly [2].

The experimental data were evaluated by impaired t-test. The results in Table 2 show that administration of Tyrode solution does indeed cause net water flux from colon to blood, and that castor oil reverses this flux. This reversal of flux by castor oil is significantly reduced by indomethacin and compound I. In a similar experiment [3], this effect of indomethacin was observed, and was accompanied by reduction in prostaglandin E (PGE) release

Table 2. Effect of pretreatment on the net water flux caused by castor oil

Fluid	Treatment	Net water flux (ml)
Tyrode	-	$= 0.707^a \pm 0.064$
Castor oil	Vehicle	$= 0.223^b \pm 0.018$
Castor oil	Aspirin	$= 0.217^b \pm 0.016$
Castor oil	Indomethacin	$= 0.150^b \pm 0.019^*$
Castor oil	Compound (1)	$= 0.160^b \pm 0.018^*$

N = 6. a: net absorption. b: net secretion. *: p = 0.05

A proposed mechanism for the cathartic action of castor oil involves the release of PGE. This is supported by the finding that ricinoleic acid in castor oil can act as an exogenous substrate for the biosynthesis of prostaglandins [5]. Ricinoleic acid also increases the amplitude of PGE elicited contractions of the guinea pig ileum, an effect which is greatly reduced by indomethacin [8]. The findings in this study therefore suggest that compound I like indomethacin, may owe its anti-inflammatory activity to its ability to inhibit PG biosynthesis.

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