

Steroidal Indoxyls: Evaluation of pK_a Values and Anti-inflammatory Activity

F.N. KAMAU^{1*}, I.O. KIBWAGE¹, G. MURIUKI², A.N. GUANTAI², H. CHEPKWONY³, J. HOOGMARTENS⁴, E. ROETS⁴ AND R. BUSSON⁴

¹Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi, P.O. Box 19676-00202 Nairobi,

²Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, P.O. Box 19676-00202, Nairobi,

³National Quality Control Laboratory, P.O. Box 29726-00202 Nairobi,

⁴Katholieke Universiteit Leuven, Faculteit Farmaceutische Wetenschappen, Van Evenstraat 4, B-3000 Leuven, Belgium.

Three steroidal indoxyls, 3-oxo-16,17-seco-16-nor-1,4-androstadien-15-(7'-methoxy-2-indoxyliden)17-oic acid, 1-(2'-indoxyliden)-2-nor-1,2-secocholestan-3-oic acid and 1-(5'-chloro-2-indoxyliden)-2-nor-1,2-secocholestan-3-oic acid were synthesized and screened for anti-inflammatory activity. Their pK_a values were also determined using a solubility method. The first compound, 3-oxo-16,17-seco-16-nor-1,4-androstadien-15-(7'-methoxy-2-indoxyliden) 17-oic acid, had an ED_{50} value of 15.3 mg/kg and a pK_a of 7.09. The cholestane derivative, 1-(2'-indoxyliden)-2-nor-1,2-secocholestan-3-oic acid, and its chloro analogue 1-(5'-chloro-2-indoxyliden)-2-nor-1,2-secocholestan-3-oic acid had ED_{50} values of 16.2 and 22.8 mg/kg, while their pK_a values were 6.56 and 7.07, respectively, suggesting that these compounds are relatively weak acids.

Key Words: Steroidal indoxyls, pK_a values, anti-inflammatory

INTRODUCTION

Glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) constitute the major classes of anti-inflammatory agents in current use. The majority of the NSAIDs are carboxylic acids with pK_a values in the range 4-5 [1]. The pK_a of acidic drugs determines their distribution in the body, including access to the site of action. It also determines the degree of ionization and thus the interaction of this group of drugs with the cyclo-oxygenase enzymes [2]. The anti-inflammatory activity of two steroidal indoxyls has been reported previously [3-4]. The present paper reports on the anti-inflammatory activity of other steroidal indoxyls, as well as an evaluation of their pK_a values for comparison with those of NSAIDs.

MATERIALS AND METHODS

Indomethacin, 4-androstadien-3,17-dione,5 α -cholestan-3-one and carrageenan type IV, were obtained from Sigma Chemical Company (St. Louis, MI, USA) while 2-nitrobenzaldehyde, 3-methoxy-nitrobenzaldehyde and 5-chloro-2-nitrobenzaldehyde were obtained from Aldrich Chemical Company. The rest of the reagents were laboratory reagent grade (BDH, Poole,

UK). Melting points were determined with a Gallenkamp melting point apparatus (London, UK).

IR spectra were run as Nujol mulls on a Perkin Elmer 727B IR recording spectrophotometer (Perkin Elmer, Buckinghamshire, UK). Ultraviolet spectra in methanol were run on a Philips PU 8740UV/VIS scanning spectrophotometer. Liquid chromatography (LC) was carried out using an LC system consisting of a L-6200 intelligent pump (Merck-Hitachi, Darmstadt, Germany), an Autosampler Spectra Series AS 100 equipped with a 100 μ l loop (Thermo Separation Products, Fremont, USA) a variable wavelength Spectra 100 UV-VIS detector set at 262 nm and an integrator model HP 3396A series (Hewlett-Packard, Avondale, PA, USA). An XTerra[™] RP C18 column (250 x 4.6 mm ID) (Waters, Milford MA, USA) was immersed in a water bath at 45 °C. For 3-oxo-16,17-seco-16-nor-1,4-androstadien-15-(7'-methoxy-2-indoxyliden)17-oic acid (I), the mobile phase used was acetonitrile-water-0.2 M ammonium acetate buffer pH 6.5 (30:65:5 v/v) while for 1-(2'-indoxyliden)-2-nor-1,2-secocholestan-3-oic acid (II) and 1-(5'-chloro-2-indoxyliden)-2-nor-1,2-secocholestan-3-oic acid (III) the ratio was

* Author to whom the correspondence may be addressed.

altered to 80:15:5 v/v and the samples were dissolved in methanol.

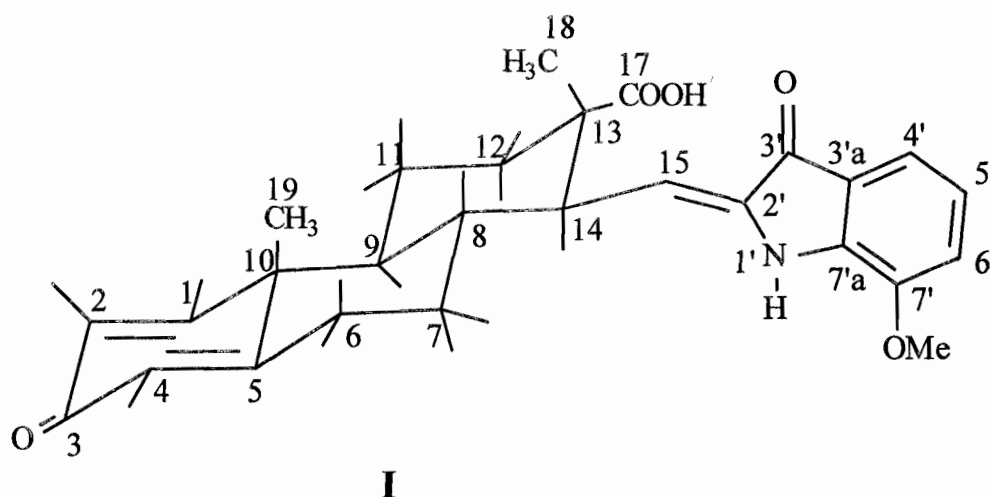
The LC-MS spectra were obtained using a Finnigan LCQ Ion Trap Mass spectrometer (Finnigan MAT, San Jose, CA, USA) with Atmospheric Pressure Chemical Ionisation (APCI) probe in the negative ion mode. The LC apparatus consisted of a Spectra System AS P1000XR quaternary pump, a Spectra Series AS 100 UV-VIS detector set at 262 nm, all from Thermo Separation Products (Freemont, CA, USA) and an integrator model 3390A (Hewlett-Packard, Avondale, PA, USA). The XTerratm RP C18 column (3.2 μ m, 100 x 2.1 mm ID) (Waters, Milford, MA, USA) was immersed in a waterbath at 45 °C. The mobile phases used were the same as those given under LC. The samples were dissolved in methanol.

¹H and ¹³C NMR spectra were recorded on a Varian (Palo Alto, USA) Unity 500 spectrometer operating at 499.193 MHz for ¹H and at 125.534 MHz for ¹³C, and using an inverse 5 mm broad-band probe with $\pi/2$ pulses of 5.5 and 18.5 μ s, respectively and equipped with pulsed magnetic field gradient coils. The standard Varian software Vnmr version 6.1b was used throughout. The measurements were performed in deuterated DMSO solution at 27 °C with tetramethylsilane as internal standard set at 0 ppm for ¹H spectra, and using the solvent multiplet set at δ 39.6 ppm for the ¹³C

NMR spectra. Spectral assignments (s = singlet, d = doublet, t = triplet, q = quadruplet, br = broad, overl = overlapped signal) were based not only on chemical shift rules and coupling patterns (using first order analysis), but also on APT (Attached Proton Test) or DEPT (distorsionless enhancement through polarization transfer) experiments for ¹³C and especially on routine 2D-correlations such as COSY45-GHSQC- (single bond or ¹J C,H-correlations), and GHMBC-experiments (multiple bond or ³J/²J C,H-correlations).

3-oxo-16,17-seco-16-nor-1,4-androstadien-15-(7-methoxy-2-indoxyliden)-17-oic acid I. To a solution of 1,4-androstadien-3,17-dione (4.26 g) in 300 ml of ethanol, 4 ml of an aqueous solution of KOH (4 g) was added, followed by a solution of 3 g of 2-nitroenzaldehyde in 100 ml of ethanol. The reaction mixture was allowed to stand at room temperature for 24 h then concentrated under reduced pressure to about 20 ml, acidified with dilute HCl and the orange precipitate collected by filtration, washed with water and dried. Re-crystallization from ethanol gave compound **I** as orange crystals (3.17 g, 47 %), m.p. 290 - 292 °C.

IR (cm⁻¹): 3350 (OH, NH), 2700-2435 (COOH), 1660, 1630 (α , β -unsaturated C=O and C=C), 1600. UV λ_{max} (nm): 248, 275, 300, 443. % purity (LC) 99.28 as partially resolved isomeric mixture (51.72: 47.56). [M-H]⁻ (LC-MS): 446 (100).



^1H NMR (DMSO- d_6): δ 1.210 (s, 3H, 19- H_3), 1.231 (s, 3H, 18- H_3), 1.23 (overl m, 2H, 7- H_a , and 9-H), 1.601(dq, 1H, $J_d=3.4$ Hz and $J_q=13.2$ Hz, 11- H_a), 1.70 (overl m, 2H, 7- H_e , and 12- H_e), 1.85 (overl m, 3H, 8-H, 11- H_e , and 12- H_a), 2.243 (dt, 1H, $J_d=13.2$ Hz and $J_t=3.2$ Hz, 6- H_e), 2.414 (dt, 1H, $J_d=4.9$ Hz and $J_t=13.2$ Hz, 6- H_a), 3.085 (t, 1H, $J=11.3$ Hz, 14-H), 3.871 (s, 3H, OMe), 5.708 (d, 1H, $J=11.7$ Hz, 15-H), 5.964 (t, 1H, $J=1.4$ Hz, 4-H), 6.148 (dd, 1H, $J=2.0$ Hz and $J=10.2$ Hz, 2-H), 6.774 (t, 1H, $J=7.7$ Hz, 5'-H), 7.094(d, 2H, $J=7.7$ Hz, 4'-H and 6'-H), 7.236 (d, 1H, $J=9.8$ Hz, 1-H), 8.878 (s, 1H, NH), 12.115 (s, 1H, COOH) ppm.

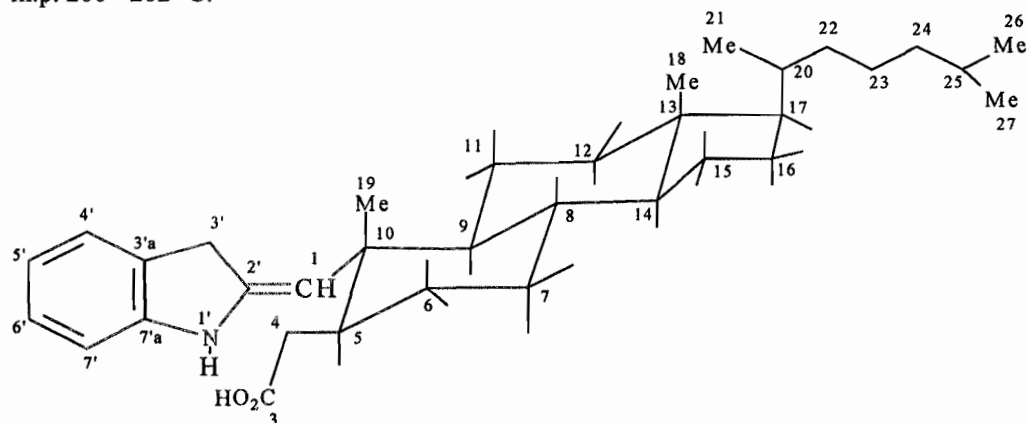
^{13}C NMR (DMSO- d_6): δ 184.9 (CO, C3), 184.7 (CO, C3'), 178.2 (COOH, C17), 169.5 (C, C5), 156.0 (CH, C1), 145.5 (C, C7'), 144.6 (C, C7'a), 138.5 (C, C2'), 127.0 (CH, C2), 122.9 (CH, C4), 121.2 (C, C3'a), 119.1 (CH, C5'), 116.5 (CH, C4'), 115.6 (CH, C6'), 114.9 (CH, C15), 55.6 (CH₃, OMe), 50.1 (CH, C9), 45.9 (C, C13), 45.1 (CH, C14), 43.2 (C, C10), 35.8 (CH₂, C12), 35.2 (CH, C8), 32.9 (CH₂, C7), 31.8 (CH₂, C6), 21.3 (CH₂, C11), 18.5 (CH₃, C19), 15.4 (CH₃, C18) ppm.

1-(2-Indoxyliden)-2-nor-1,2-seco-cholestan-3-oic acid II. About 2.5 g of 5 α -cholestan-3-one was dissolved with warming in 150 ml of ethanol. After cooling, 2 ml of an aqueous solution of KOH (2 g) was added followed by a solution of 1.5 g of nitrobenzaldehyde in 10 ml of ethanol. The solution was allowed to stand at room temperature for 72 hours. The rest of the procedure was as for the synthesis of compound I. Re-crystallization from methanol gave light-yellow needles of compound II (2.7 g, 80 %), m.p. 200 - 202 °C.

IR (cm⁻¹): 3500 (OH), 3375 (NH), 2675 - 2350 (COOH), 1720 (C=O of COOH), 1650, 1620 (α , β -unsaturated C=O and C=C), 760, 710. UV λ_{max} (nm): 222, 227, 235 (sh), 258, 300 (sh), 448. % purity (LC) 74.21. [M-H]⁻ (LC-MS): 518 (100).

^1H NMR (DMSO- d_6): δ 0.628 (s, 3H, 18- H_3), 0.822, 0.828, 0.828 (3x d, 3x 3H, $J=6.6$ Hz, 21- H_3 , 26- H_3 , 27- H_3), 1.171 (s, 3H, 19- H_3), 0.94-1.36 (overl m, 17H, 6-,7-,11-,12-,15-,16- H_a , 22-,23-,24- H_{a+e} , and 8-,9-,14-,17-,20-H), 1.405 (dm, 1H, $^2J=12.7$ Hz, 15- H_e), 1.479 (nonet, 1H, $J=6.6$ Hz, 25-H), 1.539 (m, 1H, 16- H_e), 1.610 (dm, 2H, $^2J_d=10.0$ Hz, 6- H_e and 7- H_e), 1.770 (m, 1H, 11- H_e), 1.826 (dm, 1H, $^2J_d=12.7$ Hz, 12- H_e), 1.935 (overl m, 2H, 4- H_a and 5-H), 2.119 (d, 1H, $^2J=12.9$ Hz, 4- H_e), 5.500 (s, 1H, 1-H), 6.822 (t, 1H, $J=7.6$ Hz, 5'-H), 7.074 (d, 1H, $J=8.0$ Hz, 7'-H), 7.459 (t, 1H, $J=7.7$ Hz, 6'-H), 7.500 (d, 1H, $J=7.6$ Hz, 4'-H), 8.851 (s, 1H, NH), 11.985 (s, 1H, COOH) ppm.

^{13}C NMR (DMSO- d_6): δ 185.4 (CO, C3'), 174.1 (COOH, C3), 154.4 (C, C7'a), 136.2 (C/CH, C2'/C6'), 124.0 (CH, C4'), 123.3 (CH, C1), 119.8 (C, C3'a), 118.8 (CH, C5'), 112.4 (CH, C7'), 55.7 (CH, C17), 55.6 (CH, C14), 51.4 (br CH, C9), 43.4 (br CH, C5), 42.4 (br C, C10), 42.3 (C, C13), 39.4 (CH₂, C12), 39.0 (CH₂, C24), 37.2 (CH₂, C4), 35.6 (CH₂, C22), 35.1 (CH, C20), 34.1 (CH, C8), 30.6 (CH₂, C7), 27.8 (CH₂, C11), 27.4 (CH, C25), 26.3 (CH₂, C6), 23.7 (CH₂, C16), 23.3 (CH₂, C15), 23.2 (CH₂, C23), 22.6 (CH₃, C26), 22.4 (CH₃, C27), 18.5 (CH₃, C21), 12.2 (br CH₃, C19), 11.9 (CH₃, C18) ppm.



II 5' = H

III 5' = Cl

1-(5'-Chloro-2-indoxyliden)-2-nor-1,2-seco-cholestan-3-oic acid III. About 2.5 g 5 α -cholestan-3-one was dissolved with warming in 300 ml of ethanol. After cooling, 3 ml of aqueous KOH (3 g) was added, followed by a solution of 1.5 g of 5-chloro-2-nitrobenzaldehyde in 50 ml of ethanol. After 72 hours at room temperature, the compound was isolated as described for compound I. Recrystallization from ethyl acetate gave compound III as yellow needles (3.09 g, 86 %), m.p. 205 - 207 °C.

IR (cm⁻¹): 3375 (OH), 3200 (NH), 2700 - 2450 (COOH), 1720 (C=O of COOH), 1700, 1620 (C=O and C=C of α , β -unsaturated carbonyl), 1610, 720. UV λ_{max} (nm): 244, 261, 285, 310 (sh), 453. % Purity (LC) 98.34. [M-H] (LC-MS): 552.5 (100).

¹H NMR (DMSO-d₆ at 65 °C): δ 0.648 (s, 3H, 18-H₃), 0.842, 0.846, 0.849 (3x d, 3x 3H, J=6.6 Hz, 21-H₃, 26-H₃, 27-H₃), 1.184 (s, 3H, 19-H₃), 0.94-1.36 (overl m, 17H, 6-, 7-, 11-, 12-, 15-, 16-H_a, 22-, 23-, 24-H_{a+e}, and 8-, 9-, 14-, 17-, 20-H), 1.420 (dd, 1H, J=3.2 Hz and ²J=13.2 Hz, 15-H_e), 1.506 (nonet, 1H, J=6.6 Hz, 25-H), 1.558 (m, 1H, 16-H_e), 1.643 (dm, 2H, ²J_d=11.2 Hz, 6-H_e and 7-H_e), 1.786 (m, 1H, 11-H_e), 1.841 (dm, 1H, ²J_d=12.7 Hz, 12-H_e), 1.941 (overl m, 2H, 4-H_a and 5-H), 2.132 (d, 1H, ²J=12.2 Hz, 4-H_e), 5.592 (s, 1H, 1-H), 7.116 (dd, 1H, J=0.6 Hz and J=8.4 Hz, 7'-H), 7.463 (dd, 1H, J=2.3 Hz and J=8.4 Hz, 6'-H), 7.481 (dd, 1H, J=0.6 Hz and J=2.3 Hz, 4'-H), 8.891 (br s, 1H, NH), 11.730 (br s, 1H, COOH) ppm.

¹³C NMR (DMSO-d₆ at 65 °C): δ 183.9 (CO, C3'), 173.5 (COOH, C3), 152.5 (C, C7'a), 136.2 (C, C2'), 135.4 (CH, C6'), 124.5 (CH, C1), 122.9 (C, C5'), 122.8 (CH, C4'), 120.8 (C, C3'a), 113.9 (CH, C7'), 55.6 (CH, C17), 55.5 (CH, C14), 51.3 (CH, C9), 43.3 (CH, C5), 42.5 (C, C10), 42.1 (C, C13), 39.3 (CH₂, C12), 38.7 (CH₂, C24), 37.0 (CH₂, C4), 35.4 (CH₂, C22), 34.8 (CH, C20), 33.9 (CH, C8), 30.4 (CH₂, C7), 27.4 (CH₂, C11), 27.1 (CH, C25), 26.2 (CH₂, C6), 23.4 (CH₂, C16), 23.1 (CH₂, C15), 23.0 (CH₂, C23), 22.3 (CH₃, C26), 22.1 (CH₃, C27), 18.3 (CH₃, C21), 12.3 (CH₃, C19), 11.6 (CH₃, C18) ppm.

ANTI-INFLAMMATORY ACTIVITY

The carrageenan-rat paw oedema method [5] was used. Indomethacin and compounds I, II and III were suspended in 2 % carboxymethylcellulose. Carrageenan 1 % was prepared in normal saline. The test substances were administered intraperitoneally in dose volumes of 0.5 ml into male albino rats (180-210 g), one hour before injection of 0.1 ml of carrageenan. Carrageenan was injected into the subplantar area of the left hind paw. The doses employed were indomethacin 10.00, 5.00 and 3.00; compound I 50.00, 25.00, 5.00; compound II 20.00, 10.00, 3.33; compound III 50.00, 25.00, 8.00; all doses were in mg/kg body weight and 6 rats were used per test substance dose. Control animals, six in number, received 0.5 ml of the vehicle 1 hour before the injection of carrageenan. After carrageenan injection, the initial paw 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 (ΔV_c) and test (ΔV_t) was calculated as follows:

$$\begin{aligned}\Delta V_c &= V_f - V_i \\ \Delta V_t &= V_f - V_i\end{aligned}$$

The % inhibition of oedema was calculated from the equation:

$$\% \text{ inhibition} = 100 \left[1 - \frac{\Delta V_t}{\Delta V_c} \right]$$

DETERMINATION OF pK_a VALUES [6]

The isosbestic point for each compound was determined by running UV spectra of solutions of identical concentration in methanol 0.1M HCl and 0.1M NaOH. The isosbestic points were 268 nm, 256 nm and 262 nm for compounds I, II and III respectively. A calibration was performed at the isosbestic wavelength from several dilutions in methanol.

The intrinsic solubility S_i was determined as follows: to 10 mg of the compound in a 15 ml stoppered centrifuge tube was added 10 ml of 0.1M HCl and the tube agitated for 3 h then centrifuged for 15 min; the absorbance of the supernatant was read. The mean of six such readings was taken to give the average

absorbance, from which the intrinsic solubility was read from the calibration plot.

The solubility in buffers of different pH values (S_0) was determined as follows: using 10 ml of McIlvaine buffer of different pH values, the procedure for the determination of S_i was repeated to obtain the solubility, S_0 , at each pH value. The pK_a was calculated from the following equation $pK_a = pH - \log (S_0/S_i - 1)$. Eight determinations were performed for each compound and the mean pK_a calculated. The values obtained were 7.09 ± 0.02 , 6.56 ± 0.3 and 7.07 ± 0.1 (mean \pm s.d) for compounds **I**, **II** and **III**, respectively.

RESULTS AND DISCUSSION

Synthesis of compounds **I**, **II** and **III** was achieved through the key step of condensing the starting 17- (**I**) or 3- (**II** and **III**) ketosteroid with 2-nitrobenzaldehyde **II**, 3-methoxy-2-nitrobenzaldehyde **I**, or 5-chloro-2-nitrobenzaldehyde **III**. The synthesis of steroidal indoxyls from 17-ketosteroids and 2-nitrobenzaldehyde has been reported previously [7]. A similar synthesis from a 3-ketosteroid has also been reported [8]. Support for the structures of the steroidal indoxyls **I**, **II** and **III** was from spectral data. Compounds **I**, **II** and **III** showed in their UV spectra features of highly conjugated systems. The IR spectra of these compounds exhibited absorption bands due to OH and NH groups around 3400 cm^{-1} , COOH at $2750 - 2400\text{ cm}^{-1}$ and 1700 cm^{-1} and due to α,β -unsaturated ring ketone at around 1680 cm^{-1} and 1640 cm^{-1} . In particular, peaks at 3400 cm^{-1} and 1610 cm^{-1} are characteristic of indoxyls. [9].

Definite and strong confirmation of the proposed structures was obtained from the NMR spectra. The substitution pattern in the aromatic moieties was easily deduced from the coupling information in the aromatic portion of the ^1H NMR spectrum. Supplementary information from the aliphatic region, apart from the methyl singlets, was much lower since there was a lot of overlapping of the peaks between 1 and 3 ppm, but the overall pattern and the integration ratios were very helpful for the analysis of the ^{13}C -spectra, and the few coupling constants measured still allowed for the confirmation of some typical structural features and the

deduction of the conformation of the molecules. From the analysis of the protonation degree of the peaks in the carbon spectra and the number of exchangeable protons, we could readily deduce the molecular formulas $\text{C}_{27}\text{H}_{29}\text{NO}_5$ **I**, $\text{C}_{34}\text{H}_{49}\text{NO}_3$ **II** and $\text{C}_{34}\text{H}_{48}\text{NO}_3\text{Cl}$ **III** corresponding with the data of the mass spectra. Thus we could easily show that compounds **II** and **III** are really very similar and differ only by substitution of a chloro group for the 5'-hydrogen in the indole moiety of **III**. In a similar way, the presence of a methoxy substituent in the 7'-position of the indoxyl of **I**, and the 3-oxo-cyclohexadienyl ring in going from **I** was clearly and straightforwardly confirmed from the carbon spectrum. For a reliable and unequivocal analysis of the ^{13}C spectra, all the signals as well in the ^{13}C - as in the ^1H -NMR spectra were nicely correlated with each other via 2D COSY- and GHSQC-experiments, and the whole analysis was then finally checked by a GHMBC- or multiple bond C,H-correlation experiment. For compound **III**, there was a solubility problem, which caused the molecule to show a number of very broad signals in the spectrum. To avoid this problem, all the spectra for this compound were taken at 65°C (as is indicated in the data of the experimental part) at which temperature an almost normal spectrum was obtained.

The ED_{50} (50 % inhibition) values obtained from the plot were 5.8, 15.3, 16.2 and 22.8 mg/kg for indomethacin and compounds **I**, **II** and **III** respectively. Among the steroidal indoxyls, compounds **I** and **II** were the most active. Compound **III** was the least active. The log-dose response curves for indomethacin and compounds **I** – **III** are not parallel and this may suggest different mechanisms of anti-inflammatory action for indomethacin and the steroidal indoxyls. The ED_{50} of 5.8 mg/kg obtained for indomethacin compares with the value of 5.2 mg/kg reported in the literature [1].

The carrageenan assay does detect useful anti-inflammatory substances. This method provides information of various kinds. It sheds light on the potency of the substance and it also conveys an initial impression of tolerability, insofar as it is to some extent possible, by observing the behavior of the rats, to deduce whether or not side effects occur within the pharmacologically active dose range. No deaths or side effects were observed during the screening of compounds **I**, **II** and **III**. From the results of an

experiment such as this, one can gain a rough idea as to the size of dose which would be required to elicit an effect in man, though of course, only a very approximate correlation exists between the potency observed in animal experiments and the therapeutic activity actually exerted in patients. It was found [10] for example that the plasma concentrations of phenylbutazone required to inhibit carrageenan induced oedema in the rat were similar to those required for symptomatic relief of rheumatoid disease in man.

The pK_a values for compounds **I**, **II** and **III** were found to be 7.09 ± 0.02 , 6.56 ± 0.3 and 7.07 ± 0.1 respectively making them relatively weak acids. pK_a values can be determined using partition and solubility methods. [6, 10]. The values obtained for these compounds compare with those of structurally similar carboxylic acids such as abietic acid (7.62) [11], cholic acid (6.4) and deoxycholic acid (6.58) [12]. The pK_a values for the acidic NSAIDs indomethacin, phenylbutazone and naproxen are 4.2, 4.8 and 4.5 respectively [1] making these drugs moderately strong acids that are 100 % ionized at physiological pH. It may be expected that compounds **I**, **II**, and **III** which are relatively weak acids would only be partially ionized at physiological pH. The acidity of NSAIDs that are strong acids has been implicated in their damage to mucosa cells [13]. On the other hand, weakly acidic NSAIDs such as nimesulide, celecoxib and rofecoxib are less destructive on the gastric mucosa [14].

ACKNOWLEDGEMENTS

Financial support for this work by the Deans' Committee, University of Nairobi and by the Belgian Government (VLIR grant no. Hoogmartens 107 is gratefully acknowledged.

REFERENCES

[1] F.J. Wagenhouser (Ed). Chronic Forms of Polyarthrititis, Hans Huber Bern, Stuttgart and Vienna. 1976 p. 269.

- [2] T.Y. Shen and P. Gund, *J. Med. Chem* 20 (1977) 1146
- [3] F.N. Kamau, I.O. Kibwage, G. Muriuki, A.N. Guantai, J. Hoogmartens E. Roets, C. Govaerts and R. Busson, *East Cent. Afr. J. Pharm. Sci.* 5 (2002) 44 - 48.
- [4] F. N. Kamau, I.O. Kibwage, A.N. Guantai. G. Muriuki and R. Munenge, *East Cent. Afr. J. Pharm Sci.* 6 (2003) 26 - 29
- [5] V.A. Glushkov, L.V. Amikina, Yu B. Vikhavev, O.G. Stryapunina, Yu V. Shklyayev and A.G. Tolstikov. *Russian Pharmaceutical Chemistry Journal (English Translation)* 38 (2004) 86.
- [6] Adrien Albert and E. P. Serjeant, *The Determination of Ionisation Constants, A Laboratory Manual.* Chapman and Hall, London. 1971
- [7] A. Hassner, M.J. Haddadin and P. Catsoulcos *J. Org. Chem.* 31 (1966) 1363.
- [8] F. N. Kamau, *East Cent. Afr. J. Pharm. Sci.* 8 (2005) 45 - 49
- [9] B. Witkop and J.G. Patrick *J. Am. Chem. Soc.* 73 (1951) 2188.
- [10] W.M. Kofi - Tsekpo, *Drug. Experi. Clin. Res.* 6 (1980) 421 - 423.
- [11] E.Back and B. Sternberg, *Acta Chim. Scand.* 4 (1950) 810.
- [12] M. WindHolz, *Merc Index*, Merc and Co. Inc. Rahway 1976 p.
- [13] K.D. Rainsford, J. Watkins and M. J. H. Smith, *J. Pharm. Pharmacol.* 20 (1968) 941.
- [14] G.A. Fitzgerald and C. Patrono, *N. Engl. J. Med.* 345 (2001) 433.