Synthesis of N-Substituted indoxyls and pharmacological screening of 3-acetoxy-N-methylinole-2-carboxylic acid

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ملخص

تم في هذا البحث حلقنة مركبات ٢- كربوكسي ميثيل حامض الخليك المحتوية على مجموعات مختلفة على ذرة النيتروجين وذلك لتحضير مركبات إندوكسيل.

وتم فصل مركب ٣-أسيتوكسي - ميثيل إيندول - ٢-حامض الكربوكسليك الذي تم دراسة خواصه الإقربازينية وهي الخواص المهدئة ومضادة الالتهاب.

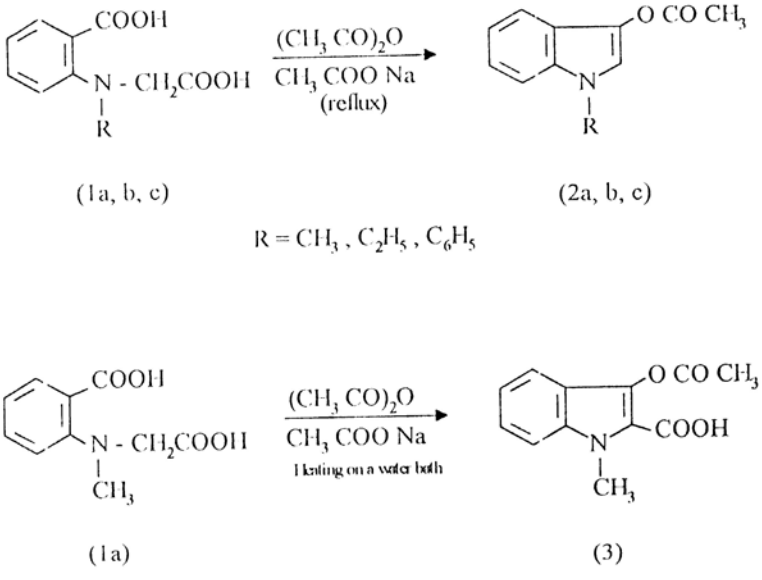
ABSTRACT

Cyclisation of some N-Substituted-N-(2-Carboxyphenyl) aminoacetic acids yielded the corresponding N-Substituted indoxyls. The analgesic and the anti-inflammatory activities of 3-acetoxy-N-methylindole-2-carboxylic acid were investigated.

Introduction:

Indoxyls may be synthesised by the cyclisation of the appropriate phenylaminoacetic acid or anthranilic acid derivatives. Cyclising agents for the former include sodium or potassium hydroxide at 260 ^{0}C[1], or sodamide with cyanide moderators[2], however, the indoxyl once formed has a great tendency to be

oxidised to indigo unless air is vigoroulsy excluded from the reaction and workup procedures. Yields also tend to be low. Cyclisation of anthranilic acids proceeds smoothly using acetic anhydride and sodium acetate at reflux temperatures[3,4], however, the preparation of N-substituted anthranilic acids is time consuming. In the present work, the cyclisatioon of some N-substituted-N-(2-carboxy-phenyl)aminoacetic acid from N-substituted anthranilic acids (Ia, b, c) was achieved by boiling under reflux for one hour in the presence of equal weights of anhydrous sodium acetate and ten times its weight of acetic anhydride. However, when the reaction was repeated using the same reagents but heating on a water bath for 30 minutes; compound (1a) gives an unexpected product. This product was found to be 3-Acetoxy-N-methylindole-2-carboxylic acid (3) whose pharmacological activities were investigated.



(Fig. 1)

Materials and Methods:

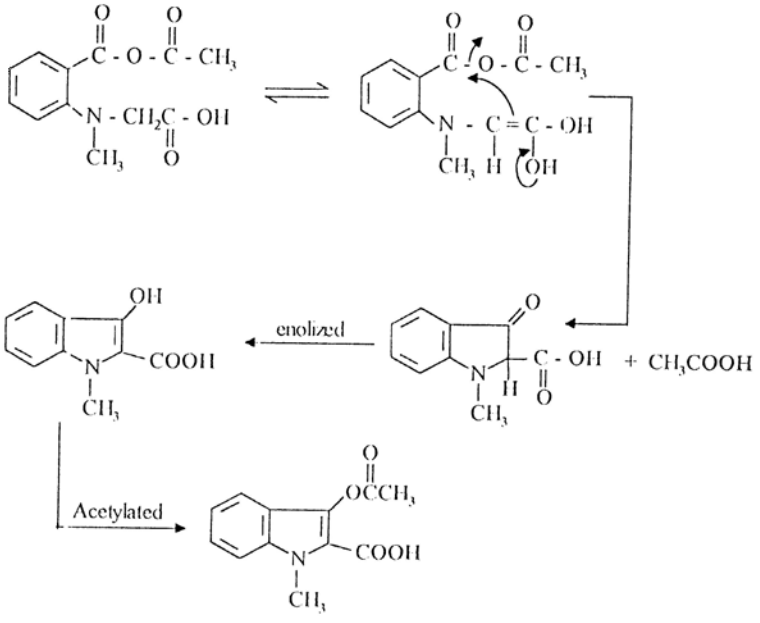
Materials:

For the synthesis of the title compounds, anthranilic acid, phenylamino acetic acid, zinc chloride, glacial acetic acid, acetic anhydride and polyphosphoric acid were purchased from BDH. For the pharmacological screening, codeine, morphine, phenobarbital and paracetamol were purchased from BDH.

Methods:

a. Chemistry:

Cyclisation of (1a) with an equal weight of anhydrous sodium acetate and a ten fold volume of acetic anhydride under reflux gave a 69% yield of N-methyl-3-acetyl-indoxyl (2a), melting point 56.5 ^{0}C, indentical with that prepared from the corresponding anthranilic acid[4]. The reaction was monitored by TLC and the optimal time for refluxing was found to be 3.5 hours, a variation of $\pm$ 10% of this time gave substantially lower yields. But when (1a) was cyclised using acetic anhydride and anhydrous sodium acetate and on heating the mixutre gently on a water-bath for 30 minutes; the 3-acetoxy-N-methylindole (2a) was not obtained. However, under the mild experimental conditions, decarboxylation did not take place and indole acetic acid derivative (3) was obtained as shown in (Fig. 2).



(3)

(Fig. 2)

b. Pharmacology:

(i) - Anti-pyretic activity:[5]

The hind-paw edema of Wistar rats (180-200 gm) were used to investigate the anti-pyretic activity of the compound. Hind-paw edema was induced by a single injection of 0-1 yeast in normal saline into the right hind-paw. An equal volume of normal saline was injected into the left hind-paw as control. The volumes of both hind-paws of each rat were measured by means of a plethysmometer (model 7150, Ugo Basile).

(ii) - Analgesic activity:

The analgesic activity of the compound was tested using two methods namely the direct hot plate at 55 ^{0}C and the writhing test as descibed by Nodine.[6]

(iii) - Acute toxicity:

The toxicity was assessed by administering different doses of the compound ranging from 50 mg/kg suspended in Arabic gum vehicle 20% (W/V) to different batches of 10 male rats each weighing 180$\pm$ 20g each. The LD_{50} was calculated according to lichfield and Wilcoxon.[7]

Results and Discussion:

Chemistry:

The microanalytical analysis of the indole acetic acid derivative (3) gives it a molecular formula $C_{12}H_{11}NO_4$ (Table 1). The product gives effervescence on treatment with sodium bicarbonate solution, indicating the presence of a carboxyl group. The IR spectra shows two carbonyl bands at 1763 and 1720 cm^{-1}. The PMR spectrum shows two singlets at δ 3.71 and δ 4.00 integration for three protons each (N-CH_3) and ($OCOCH_3$) respectively. Based on these chemical, microanalytical and spectroscopic data (Table 2), the compound was assigned the structure 3-acetoxy-N-methylindole-2-carboxylic acid (3). Successful cysclisation of (1a) were also achieved using a mixture of zinc chloride, glacial acetic acid and acetic anhydride[8] under reflux for one hour and also with a mixture of polyphosphoric acid and acetic anhydride[9] under reflux for two hours. The yields of (2a) being respectively 33% and 82%.

N-Ethyl-N-Phenylaminoacetic acid hydrochloride, m.p. 183^0,

(lit.[10] m.p. 179^0) also cyclised successfully to give (2b) the yields being 59%, 24% and 63% respectively. The product was vacuum distilled, then boiled with petroleum ether (40-60) to yield a pale green semi-solid, whose structure was confirmed by spectroscopy and conversion to N N–diethylindigo.[11]

Pharmacological studies:

a: The LD_{50} of compound (3) was found to be more than 50mg/kg.

b: Analgesic activity:

The direct hot plate method was used to estimate the effect of 50mg/kg codeine given subcutaneously (S.C), the analgesic activity represented as reaction time. Codine was used as a reference drug to the effects of controls and the test compound (3), the last was also injected S. C in a dose of 50 mg/kg.

A comparison was made for all above readings where it was found that the effects of the test compound (3) (represented as reaction time) was 84 sec. compared with 180 sec. of codeine and 33 sec. of controls.

In another setts of experiments, the analgesic activity of the test compound S.C given in a dose of 50mg/kg was compared with that of 10 and 15mg/kg morphine S.C and those of controls. The effects of 15mg/kg morphine was 120 sec. compared with 18 sec. of controls and 120 sec. for the test compound.

Morphine as a reference drug when used in a dose of 10mg/kg S.C, the reaction time was 34 seconds. Usually codeine exerts analgesic activity which is less than that of morphine but its efficacy is quite high when it is taken orally (Harvey and champ[13]). The analgesic activity of the test compound (3) was

assessed using another technique when the acetylcholine writhing test was employed. This was represented as a percentage of the inhibition in compound (3).

In this test 100mg/kg of the test compound given I. P caused 64.4% inhibition of the total number of the writhes, compared with 46.7% inhibition of 20mg/kg codeine used also I.P.

c: Anti-pyretic activity:

Compound (3) was examined for its possible anti-pyretic effect using the rat-paw pressure test on inflamed and normal paw of the mice, these results were compared with those of 200mg/kg paracetamol in reference to 150mg/kg of compound (3). No significant anti-pyretic effect was noticed for compound (3).

Paracetamol is a remarkable anti-pyretic drug when compared with other drugs (Katzung[14]).

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Table 1
Microanalytical results of compound (3)

Compound (3)	Recrystallization Solvent	Yield (%)	Formula	Analysis					
				Calculated			Found		
				C	H	N	C	H	N
	H_2O	47	$C_{12}H_{11}NO_4$	61.80	4.75	6.01	61.79	5.18	5.99

Table (2)
Spectroscopic data of compound (3)

Compound (3)	V_{max} (KCI) cm^{-1}		δ ppm (D_6DMSO)			
	C=O	C=C	N - C - CH_3 (O)	O - C - CH_3 (O)	N - CH_3	Aromatic H
	1763, 1720	1620	3.71	4.00	7.30	7.00-7.58

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