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Synthesis And Pharmacological Activity of some Mannich Bases of Dehydrozingerone

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Mannich bases of dehydrozingerone were synthesised by the Mannich reaction with dialkylamine hydrochlorides and also by aldol condensation of vanillin with dialkylaminobutan-2-one. They displayed antiinflammatory, analgesic and antipyretic activities.

DEHYDROZINGERONE (DZ), an intermediate in the synthesis of zingerone is reported to be a better antiinflammatory agent than latter, a phenolic constituent of ginger *(Zingiber officinale)*[1]. DZ is found to inhibit histamine, prostaglandins,5-HT, dextran and bradykinin-evoked oedema. It inhibits ADP-induced platelet aggregation and nitrite induced oxidation of haemoglobin[2]. It has powerful oxygen radical scavenging effect and inhibits lipid peroxidation[3,4]. Various chemical modifications of DZ, such as alkyl,alkoxy and halogeno substitutions in the benzene nucleus have been prepared and reported to have enhanced antiinflammatory and analgesic activities. Dehydrozingerone is (E)-4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one, has been reported to possess mild analgesic and antipyretic activities without much ulcerogenic effect[6,7].

The present study was initiated to enhance the water solubility of the lead compound by preparing its Mannich bases. The introduction of pharmacophoric site such as tertiary aminoalkyl systems in DZ may alter the receptor activity and change their bioavailability of the DZ. Therefore it was decided to prepare Mannich bases of DZ to explore certain pharmacological profile. The test compounds were synthesized by two methods and compared the percentage yield. One method employed the classical Mannich reaction using DZ, secondary alkylamines hydrochloride and paraformaldehyde, The other method is a direct aldol condensation of vanillin with 4-alkylaminobutan-2-one.

The products obtained by both the methods were found to be identical but the percent yield in the aldol condensation method is found to be more. The Mannich bases exhibited fairly good antiinflammatory and analgesic activities when compared to DZ and were without any ulcerogenic property.

MATERIALS AND METHODS

Synthesis:

The Mannich bases were prepared by two methods. The percent yield was calculated with reference to vanillin utilised for the synthesis. The compounds synthesised were characterised by TLC, MP, and by IR and NMR spectral interpretation.

Method A

The starting materials were DZ, secondary alkylamine hydrochloride and paraformaldehyde. Vanillylidene acetone (DZ) was synthesised by modified Nomura method[8]. The mannich bases MB1 to MB6 were prepared from various amines like dimethylamine, diethylamine, diethanolamine, di n-propylamine, morpholine and piperidine[9].

Dehydrozingerone (0.1 mol) and respective secondary alkylamine hydrochloride (0.1 mol) were dissolved in

*For correspondence

ethanol (50 ml). The mixture was heated to boiling, paraformaldehyde (0.14 mol) was added slowly and refluxed for 30 min. The resultant solution was filtered and kept overnight. The solids separated upon cooling was recrystallised from methanol. The MP and % yield of Mannich bases prepared are given in Table 1.

Method B

Vanillin was allowed to undergo aldol condensation with 1-dialkylaminobutan-3-one. The respective butanones were prepared by a common procedure mentioned below[10].

A mixture of secondary alkylamine hydrochloride (0.2 mol), acetone (60 ml, 0.88 mol) and paraformaldehyde (8.4 g, 0.28 mol) in isopropyl alcohol (40 ml) was refluxed for 4 h on a steam bath, concentrated at reduced pressure and mixed with 50 % sodium hydroxide (80 ml). Upon cooling the mixture seperated into two layers. The Mannich base which separated as light liquid, was washed with distilled water, dried over anhydrous sodium sulphate and purified by distillation at reduced pressure.

The test compounds were prepared by stirring vanillin (0.1 mol) and respective 1-dialkylaminobutan-3-one (0.11 mol) with cold sodium hydroxide (10%, 20 ml) for 1 h. The resultant solution was neutralised with dilute acetic acid, the Mannich base separated upon cooling was washed with cold water, converted into hydrochloride and recrystallised from methanol. The purity of the compounds synthesised were determined by TLC and MP and they were characterised by IR and NMR spectra data. The important spectral features of MB1 are: IR γ_{max} (KBr) : 3237 (Ar, OH), 3200 - 2600, OH stretch, 1655 (C=O), 1581 (Ar, C=C), 1278 (-$N(CH_3)_2$, 1168(Ar, C-O), 980 (-CH=CH-)(E) : ^{1}HNMR ($CDCl_3$, 90 MH_Z) δ 2.12 (6H, s, $N(CH_3)_2$), 2.34 (3H, s, O=C-CH_2-), 3.94 (3H, s,-OCH_3), 5.34 (4H, s, 0=C-CH_2-CH_2-),6.0 (1H, s, OH), 6.52 and 7.46 (2H, each d, J=16H_Z (E) CH=CH). 6.92 (H, s, - Aromatic), 7.00 (2H, s, - Aromatic). The melting point and percent yield of each compound are given in Table 1.

BIOLOGICAL ACTIVITIES

The test compounds were water soluble and therefore sample for biological testings were prepared in distilled water. Oral toxicity (LD_{50}) was determined in albino mice in groups of 8 of either sex for each dose tested[11]. One tenth to one fifteenth of oral LD_{50} (130-200 mg/kg dose) of each compound was selected for oral for oral dose in the biological screening. 0.5 m. mol/kg dose of test compounds (125 mg for MB_1 to 155 mg for MB_3) was employed to compare the biological activities. 1 m, mol/kg was employed for ulcerogenic activity studies. The m. mol equivalence of compounds synthesized are given in Table 1.

Antiinflammatory Activity:

The test was performed by the technique of Winter et al[12]. The oedema was induced in albino (swiss) rats in groups of 6 by injecting 0.1 ml carrageenin (1% w/v suspension in normal saline)into subplanter region of the left hind paw. The volume of paw was measured immediately using a plethysmometer after carrageenin injection and again 3 h later. The test compounds (MB1 to MB6)and DZ in 0.5 m mol/kg dose were given orally 1 h prior to carrageenin injection. Indomethacin 5 mg/kg oral route was employed as the positive control. The mean increase in paw volume and standard error (S.E) were calculated and the results were expressed as % inhibition of oedema as compared to the control. (Table 2).

Analgesic Activity:

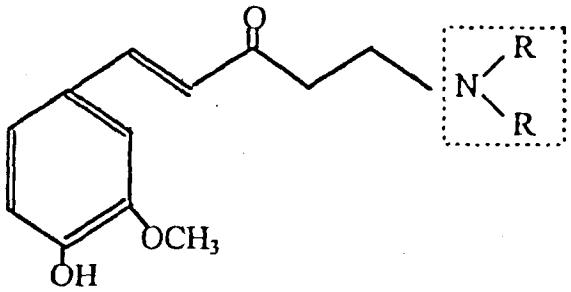
Acetic acid-induced writhing syndrome in mice was employed for studying analgesic activity[13]. The test compounds, DZ and aspirin in 0.5 m mol/kg dose were used to antagonise the writhings produced by the injection of 0.6% acetic acid in albino mice. The analgesic activity was calculated as % reduction of writhing in test animals in comparison with control. (Table 2).

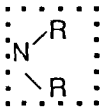
Antipyretic Activity:

The method was based on Typhoid and Paratyphoid A and B, (TAB) vaccine-induced pyrexia in albino rats[14]. The animals were made pyretic by injecting TAB vaccine 0.1 ml/rat subcutaneously. The test compounds, DZ and paracetamol were administered orally in 0.5 m mol/kg dose one hour after injection of TAB vaccine. The hourly rectal temperature(°C) was measured upto 4 h using telethermometer. The mean reduction in rectal temperature is compared with DZ and standard.

Ulcerogenic Effect: The ulcerogenic effect was determined using the method described by Cashin *et al*[15].

Table 1
Mannich bases of Dehydrozingerone



Compd		mp*	Method A % Yield **	Method B %Yield **	LD_{50} p.o g/kg @	m. Mol Equivalent
MB1	Dimethylamino	165-67	34.0	52.7	2.0	249
MB2	Diethylamino	168-69	38.1	58.8	2.0	277
MB3	Diethanolamino	143-44	35.7	53.1	2.0	309
MB4	n-Dipropylamino	172-73	40.3	59.8	2.0	305
MB5	Morpholino	158-60	33.5	56.2	2.0	291
MB6	Piperidino	162-63	31.7	49.3	2.0	289
DZ		-	-	-	3.0	192

* M. P of the compounds were taken in open capillary and were uncorrected

** The % Yield was calculated with reference to the amount of vanillin used in each procedure.

@ LD_{50} in albino mice (n=8)

Food was withheld for 16 h from each group of 6 rats (130-150 g) and the test compounds, DZ and aspirin in 1 m mol/kg were administered orally. The control group was treated with distilled water alone. Three hours after dosing the rats were killed, stomach removed, washed with normal saline and opened along the lesser curvature. Ulceration of the mucosa was examined under microscope. The severity of lesions in the mucosa was scored according to an arbitary system: 0=no lesion, 0.5 = hyperaemia, 1=one or two slight lesions present, 1.5 = more than two lesions present, 2 = severe lesions, 3=very severe lesions, 4= lesions involving the whole mucosa. The ulcerogenic scores obtained are given in Table 2.

RESULTS AND DISCUSSION

The Mannich bases prepared by both methods were found to be identical. It is interesting to note that the % yield of the Mannich base by direct Mannich reaction with DZ, secondary alkylamine hydrochloride and paraformaldehyde is less than that of the method B, where vanillin is condensed with respective dialkylamino-butanones (Table 1). The LD_{50} data indicates that the Mannich bases of the DZ are slightly more toxic than DZ in oral route. Approximately one tenth of LD_{50} was selected for testing biological activities. All the Mannich

Table 2 : Biological Activity

Compound	Antiinflammatory Activity @%±S.E.	Analgesic Activity* % Activity	Ulcerogenic Score**	Antipyretic activity Mean redn. rectal Temp°c#
Control	—	—	—	0.5 ± 0.32
MB1	62.9±4.9	45.0	0.5	2.62±0.21
MB2	65.8±5.3	49.2	0.5	2.10±0.19
MB3	52.3±6.0	51.9	0.5	2.60±0.31
MB4	52.9±5.3	47.0	0.5	1.95±0.18
MB5	60.4±5.1	52.9	0.5	1.65±0.20
MB6	40.7±3.9	33.1	0.5	1.00±0.26
DZ	38.6±3.1	30.1	0.5	0.85±0.16
+ cont1	86.3±6.3	74.8	2.0	3.05±0.31

@ Test Compounds in 0.5 m mol/kg p.o: Positive control Indomethacin 5 mg/kg p.o Carrageenin-induced paw oedema, n=6, p=< 0.05 (Student 't' test)

* Test Compounds in 0.5 m mol/kg p.o; Positive control Aspirin 0.5 m mol/kg p.o., Acetic acid-induced writhing in albino mice, n-6, p=<0.05 (Student 't' test)

** Test compounds in 1 m mol/kg p.o; Positive control Aspirin 1 m mol/kg p.o., arbitary score as per cashin et al method, n=6, p=<0.05)Student 't' test)

\# Test compounds in 0.5 m mol/kg p.o; Positive control paracetamol 0.5 mol/kg p.o. Mean reduction of hyperpyrexia, induced by TAB vaccine in albino rats, n=6, p=<0.05)Student't' test)

basesexhibited fairly good antiinflammatory activity when compared to the parent compound DZ. Among the test compounds, the dimethylamino, diethylamino and morpholino derivatives (MB1, MB2, MB5) showed higher activity than the others. All the test compounds exhibited marked analgesic activity, in particular MB5 and MB3. The antipyretic activity was calculated as mean change in rectal temperature (°C) of TAB vaccine-induced hyperpyrexia in albino rats. Compounds MB1 and MB3 showed the best antipyretic activity than DZ. The results clearly indicates that the test compounds, unlike DZ exerts predominant inhibition of inflammatory mediators from phlogogenic stimuli. The Mannich bases exhibited very low ulcerogenic score and are comparable with that of the parent compound DZ. The over all results indicate that the Mannich bases of DZ are good antiinflammatory agents in acute experimental models with very low ulcerogenic effect. It is worth to consider the compounds as leads for further development or as candidates for detailed toxicological, pharmacological and biochemical studies as antiinflammatory agents.

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