Acta Poloniae Pharmaceutica – Drug Research, Vol. 64 No. 2 pp. 179–182, 2007 ISSN 0001-6837
Polish Pharmaceutical Society

INHIBITORY EFFECT OF 3-CARBOETHOXYPYRIDINE AND 3-CARBOBUTOXYPYRIDINE ON ISOLATED RAT UTERUS

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Abstract: 3-Carbomethoxypyridine (CMP) was isolated and characterized from the leaves of *Pyrenacantha staudtii* Hutch and Dalz, family *Icacinaceae*, in our earlier study and was found to possess an inhibitory activity on the isolated rat uterus. In order to study the structure – activity relationship, derivatives of CMP were obtained synthetically, purified and characterized by spectroscopic techniques such as infra red spectroscopy (IR), nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and mass spectrometry (MS). The synthesized compounds were subjected to pharmacological testing using isolated rat uterine preparation in oestrus suspended in an organ bath containing De Jalon physiological salt solution (PSS). The force of contraction was recorded via an isometric transducer connected to an Ugo Basile recorder. The effects of these two compounds were compared with salbutamol as a positive control. 3-Carboethoxypyridine (ECP) demonstrated very significant ($p < 0.005$) inhibitory activity on the uterus with a total elimination of the spontaneous contractility at a dose of 0.4 mg/mL. Carbobutoxypyridine (BCP) also demonstrated a marked reduction of oxytocin-induced contractions and elimination of spontaneous activity. The study lends support to the potential use of these agents as tocolytics.

Keywords: inhibitory effect, rat uterus, derivatives of 3-carbomethoxypyridine

The use of medicinal herbs in the treatment and prevention of diseases is as old as mankind. Medicinal plants are used in different areas of human endeavour. These range from traditional medicines, herbal medicines, and health food such as nutriceaticals to galenicals, phytochemicals and industrially produced pharmaceuticals. Furthermore, medicinal plants constitute a source of valuable foreign exchange for most developing countries, as they are ready source of drugs such as quinine and reserpine, of galenicals like tinctures and of intermediates (e.g. diosgenin from *Discorea sp*) in the production of semi-synthetic drugs. In the light of the forgoing, we demonstrated the inhibitory activity of the crude methanolic extract of *Pyrenacantha staudtii* leaves and various fractions of the extract on rat isolated uterine preparation (1, 2). We also reported the isolation and characterization through a bio-assay guided method one of the active compounds, 3-carbomethoxypyridine from the methanolic extract of *Pyrenacantha staudtii* Hutch and Dalz, family *Icacinaceae* (3). The compound was found to possess significant inhibitory activity on the rat isolated uterus (4).

This study was designed primarily to determine the effect of various derivatives of 3-carbomethoxypyridine on the isolated rat uterus aiming at determination of structure – activity relationship (SAR).

EXPERIMENTAL

Reaction of nicotinic acid with methanol, ethanol and butanol

A mixture of 3.7 g of pure nicotinic acid, 12 mL of respective alcohol and 5 mL of concentrated sulfuric acid was refluxed for 1 h on a steam bath. The resulting solution was cooled and transferred onto 20 g of crushed ice. Sufficient ammonia solution was added to render the solution strongly alkaline. The mixture was extracted with 25 mL portions of diethyl ether and the residue distilled under reduced pressure to give the desired compound.

Pharmacological evaluation

Determination of LD_{50} values of the compounds

The method of Lorke (5) was used. Twenty mice (20 – 22 g) of either sex were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Benin City. The animals were randomly divided into five groups of

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four mice each. Prior to testing, the animals were feed with mice pellets and had free access to drinking water but starved for 12 h before testing. The first four groups were orally administered with 1, 2, 3 and 5 g/kg of the investigated compounds. General symptoms of toxicity and mortality were first observed for 24 h after which the animals were left for further 14 days for any delayed toxicity.

Effect of the compounds on rat isolated uterus

Female Wistar rats weighing about 150-160 g were pretreated with 1mg/kg of stilbesterol 48 h prior to the actual experiment. The rats were killed by cervical dislocation and exsanguination. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1 cm piece was mounted in a 50 mL organ bath containing De Jalon solution with the following chemical composition: NaCl, 9 g/L, $NaHCO_3$, 0.5 g/L, D-glucose, 0.5 g/L, KCl, 0.402 g/L, $CaCl_2 \times 2H_2O$, 0.08 g/L at a pH of 7.35 (6, 7).

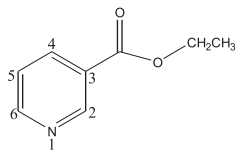
The tissue was aerated with 95% oxygen and 5 % carbon (IV) oxide at 37°C. The spontaneous contraction of the uterus was recorded with FT 03 transducer connected to an Ugo Basile recorder (7075). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500 mg). The tissue was allowed to equilibrate for 30 min before the start of the experiment.

The dose – response curves for oxytocin and various compounds (ethylpyridine 3-carboxylate – ECP, methylpyridine 3-carboxylate – MCP and butylpyridine 3-carboxylate – BCP) were determined.

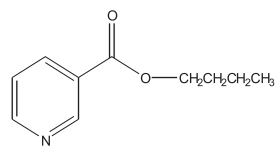
Statistical analysis

All results are expressed as the mean of four experiments ± SEM. The statistical package used was SAS, 1994. Users guide, Version 8.2. SAS Institute Inc., Cary, NC, USA. The statistical significance ($p < 0.05$) of differences between means was assessed by the analysis of variance followed by Duncan's multiple range tests.

RESULTS AND DISCUSSION



3-Carboethoxypyridine



3-Carbobutoxypyridine

Compound: 3-carboethoxypyridine (b.p. = 223-225°C, lit. 222-224°C) (8).

IR (KBr) cm^{-1}: 1720.3 (CO), 2980.5 (aromatic CH), ^{1}H-NMR: (DMSO-d_6) δ (ppm): 9.20 (1H, s, H-2), 8.8 (1H, d, H-4), 8.30 (1H, d, H-6), 7.60 (1H, dd, H-5, J = 6.2, 7.9 Hz). ^{13}C-NMR: (DMSO-d_6) ä (ppm): 164.60 (COO), 153.51(C_6), 149.86 (C_2),125.68 (C_3), 124.17 (C_5). MS: 152 (M^+ + 1), 151 (M^+) $C_8H_9O_2N$: 151.1605. Elemental analysis: calcd.: C, 63.58 %, H, 5.96 %, N, 10.21 %, found: C, 63.46 %, H, 5.92 %, N, 10.08 %.

Compound: 3-carbobutoxypyridine (b.p. = 124-125°C, lit. 122-23°C) (9), (R_f 0.57 in $CHCl_3$: CH_3OH, 1:4, v/v). IR (KBr) (cm^{-1}): 1720.3 (CO), 2958.3 (aromatic CH), ^{1}H-NMR (DMSO-d_6) δ (ppm): 9.0 (s, H-2), 8.8 (1H, d, H-4), 8.30 (1H, d, H-6), 7.50 (1H, dd, H-5, J = 6.2, 7.9 Hz). ^{13}C-NMR (DMSO-d_6) δ (ppm): 164.62 (COO), 153.50 (C_6), 149.85 (C_2), 136.65 (C_4), 125.66 (C_3), 123.79 (C_5). MS: 179 (M^+ + 1), 178 (M^+); $C_{10}H_{13}O_2N$: 179.2116. Elemental analysis: Calcd.: C, 67.04 %, H, 5.03 %, N, 10.21 %; found: C, 6.99 %, H, 5.12 %, N, 10.20 %.

Chemical reactions with Lassaigne and ninhydrin reagents suggested that ECP is a nitrogenous compound with a strong carbonyl group. This was confirmed by the IR absorption bands for carbonyl (1720.3 cm^{-1}). The MS showed an M^+ + 1 ion at m/z 151, and an M^+ at m/z 151.245 for the molecular formula $C_8H_9O_2N$. The ^{13}C-NMR spectrum showed five carbon signals with the carbonyl group appearing at 164.60 ppm. In the ^{1}H-NMR spectrum the coupling pattern of 4 aromatic protons gave rise to a singlet and doublet of doublets at 9.2 and 7.6, respectively. The MS of the second derivative – BCP, showed M^+

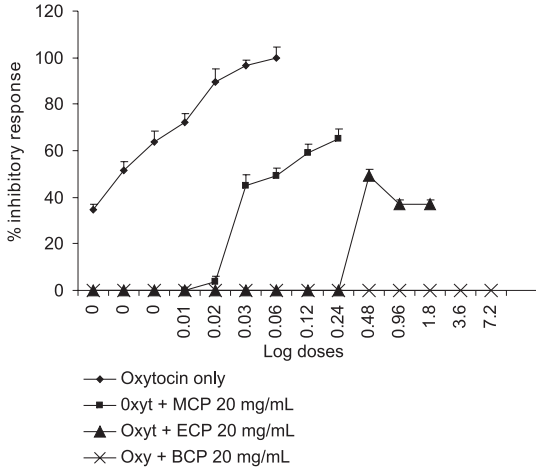
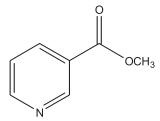
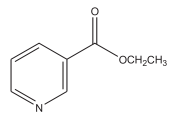
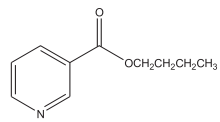
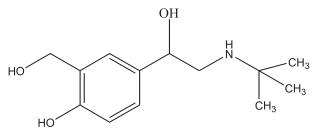


Figure1. Inhibitory activity of 3-carbomethoxypyridine (MCP), 3-carboethoxypyridine (ECP) and 3-carbobutoxypyridine (BCP) on rat isolated uterus.

Table 1. Structure-activity relationship of the investigated compounds

Compound	Dose	% Inhibition	Acute toxicity
 3-carbomethoxypyridine (MCP)	20 mg/mL	60.28 ± 1.24	5000 mg/kg
 3-carboethoxypyridine (ECP)	20 mg/mL	90.08 ± 0.45	3000 mg/kg
 3-carbobutoxypyridine(BCP)	20 mg/mL	100 ± 0.00	2000 mg/kg
 α¹-[(*tert*-butylamino)methyl]-4-hydroxy-*m*-xylene-α,α¹-diol	30 µg/mL	73.54 ± 0.35	–

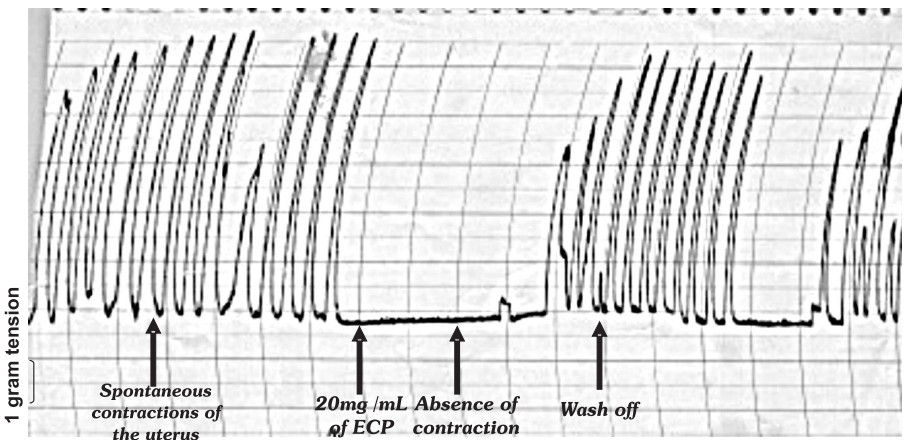


Figure 2. Effect of 3-carboethoxypyridine (ECP) on the spontaneous contraction of the uterus

at m/z 179 for the molecular formula $C_{10}H_{13}O_2N$. It also showed characteristic mass fragments at m/z 179, 164, 106, and 78, suggesting the presence of pyridine ring. The 10 carbon signals were clear in the ^{13}C-NMR spectrum, of which 5 signals were accounted for the pyridine moiety. ^{1}H-NMR spectrum revealed the presence of two CH_2 sextets and a triplet at 1.3, 1.7 and 4.2 ppm, respectively.

The LD_{50} of the compounds appeared to be partially non toxic at doses of 1-5 mg/kg (Table 1). The result of the pharmacological study showed the inhibitory activities of 3-carbomethoxypyridine, 3-carboethoxypyridine, and 3-carbobutoxypyridine on the isolated rat uterus in an organ bath containing De Jalon solution. The inhibitory activity was evaluated using the oxytocin induced contractions and the spontaneous contractility of the uterus. Hence, the percentage response of oxytocin alone, and in the presence of the various derivatives of 3-carbomethoxypyridine were determined.

3-Carbomethoxypyridine at a dose of 20 mg/mL produced a significant ($p < 0.05$) inhibitory effect on the rat uterus when compared to oxytocin alone. For instance, a dose 0.1 IU of oxytocin pro-

duced percentage response of 37% of the maximum when given alone. This was reduced to 5% in the presence of MCP and with a reduction of the spontaneous activity of the uterus, whereas 3-carboethoxypyridine showed no response with this dose of oxytocin.

For 3-carboethoxypyridine there was a significant ($p < 0.05$) and marked inhibitory effect on the oxytocin induced contractions, with a shift of the dose response curve far to the right (Fig.1), and with total elimination of the spontaneous contractions of the uterus. However, unlike 3-carbomethoxypyridine (MCP) where spontaneous contractions were restored after 4 min, the contractions of the uterus with ECP were restored 1 h after washing (Fig. 2). The implication of this is that the compound ECP will be very useful in relaxing the smooth muscle of the uterus during threatened abortion by abolishing both spontaneous contractile activity and oxytocin induced contractions of the uterus.

3-Carbobutoxypyridine (BCP) showed a significant ($p < 0.05$) inhibitory activity on the oxytocin induced contractions of the isolated rat uterus at a dose of 20 mg/mL (Fig. 1). The relaxation produced was comparable to salbutamol. Unlike the MCP and ECP, BCP completely relaxed the uterus without any spontaneous activity/contractions (Fig. 1) neither did it respond to the higher doses of oxytocin. The possible clinical implication is that it will help in the treatment of moderate to severe threatened abortion, where urgent therapeutic intervention is necessary.

It could be deduced from this study that the increase in the alkyl substituent increases the inhibitory activity of the compounds. There is a direct proportionality between the level of the alkyl moiety and the lipophilicity of compounds or drugs and ability to cross lipid membranes. This probably is responsible for the increased inhibitory activity of 3-carbobutoxypyridine.

Salbutamol still caused a significant relaxation of 73.54% on the uterus at a dose (inhibition) of 30 mg/mL, thus, confirming the potency of the drug at the smallest dose (Table 1).

Further studies need to be carried out to establish the exact mechanism of action of the compounds, and also to investigate the effects of the compounds on vital organs such as the liver, kidney and the brain. This will help in establishing the chronic toxicity profile of the compounds.

CONCLUSION

The findings from this research work revealed the inhibitory activity of the derivatives of 3-carbomethoxypyridine on isolated rat uterus, with the 3-carbobutoxypyridine showing the highest level of inhibition, followed by 3- carboethoxypyridine and then 3-carbomethoxypyridine. Though the compounds are known, their interesting tocolytic (uterine relaxant) effects are reported for the first time. These compounds could therefore be useful in the treatment of threatened abortion.

Acknowledgment

The authors are grateful to University of Benin Research Grant (URPC 2006) for this study. We also express our profound appreciation to Frau Martina Hense (Jena, Germany) for their invaluable assistance in running the spectra.

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Received: 18.09.2006