Spasmodolytic Activity of Methyl Angolensate: a Triterpenoid Isolated from *Entandrophragma angolense*

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*Entandrophragma angolense* is a medicinal plant used in folk medicine against several diseases including peptic ulcer. Methyl angolensate was isolated from *E. angolense* by recrystallization from methanol. The needle-like crystals were characterized and tested on isolated rabbit jejunum, guinea pig ileum and the rat fundus strip. The compound was also evaluated on the gastrointestinal transit in mice. The results showed that the compound exerted significant concentration dependent inhibition of smooth muscle and reduced the propulsive action of the gastrointestinal tract in mice. The relaxation observed did not attenuate acetylcholine and histamine induced contractions, but was found to inhibit contractions induced by serotonin. It is therefore suggested that methyl angolensate may exert its activity on gastrointestinal smooth muscle via serotoninergic mechanisms.

**Key words** *Entandrophragma angolense*; methyl angolensate; smooth muscle; serotonin

*Entandrophragma angolense* (Welw) C. DC. (Melicaceae) is a popular plant commonly found on the West African coast. It is an opened crowned tree that resembles African mahogany. The stem bark of *Entandrophragma angolense* is widely used in ethnomedical treatment of various gastrointestinal disorders including peptic ulcer in humans. Studies on the wood constituents of the plant have been done. 1) The antilucre activity of this plant has also been reported. 2) Another species of the genus *E. angolense* was reported to cause 100% gastroprotection in experimental ethanol-induced gastric lesion in rats. 3) The antilucre activity of methyl angolensate, a triterpenoid isolated from *E. angolense* was reported by Njar, et al. 4) Other isolated compounds from *E. angolense* include entandrolide, 14) gedunin and β-sitosterol. 15) There are no reports in the literature on the direct effects of methyl angolensate on the gastrointestinal smooth muscles and gastrointestinal transit time in mice. In this report, we present the results of the effects of methyl angolensate on these isolated smooth muscle preparations and suggest a possible mechanism of action.

**MATERIALS AND METHODS**

**Plant Material** The stem bark of *E. angolense* was collected from Owo, Ondo State, Nigeria in September, 1997. The plant was identified and authenticated by the late Mr. A. Ohaeri of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (number 3603) is deposited at the herbarium for future reference.

**Extraction and Isolation of Methyl Angolensate** The stem bark was collected, air-dried and pulverized into coarse powder using a hammer mill (Gondard & G, Paris). The powdered plant material (400 g) was extracted with 11 of n-hexane for 24 h using a Soxhlet extractor. The extract was concentrated _in vacuo_ to a solid residue with a yield of 4 g. The residue was crystallized from methanol to give methyl angolensate (2.4 g) as needle crystals. The crystals of methyl angolensate were purified and compared with the authentic standard using TLC, infrared, UV spectroscopy and melting point correlation. 5)

**Animals** Adult guinea pigs (300—400 g), New Zealand rabbits (1.5—2.5 kg), Wistar rats (180—220 g) and Swiss albino mice (18—24 g) bred in the Animal Facility Centre, NIPRD were used in these studies. The animals were kept under standard conditions of temperature (24±2 °C) and 12 h light/dark cycle. The animals were fed with standard feeds (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*.

**Drugs** Drugs used were acetylcholine chloride, histamine (free base), serotonin sulphate and atropine sulphate (all from Sigma Chemical Company, U.S.A.), mepyramine maleate (M & B) and cyproheptadine hydrochloride (MSD). All drug solution were freshly prepared. Appropriate parallel control experiments were always carried out in order to correct the possible effects caused by vehicle alone. Methyl angolensate was first triturated with tragacanth (a suspending agent) after which it was made up to the required concentration with distilled water.

**Evaluation of Pharmacological Activity. Studies on the Rat Fundus Strip** Wistar rats of either sex were used for this experiment. They were stunned and bled. The abdomen was opened and the stomach was removed. Strips 4—5 mm wide and 20 mm long were made and set up in a 20 ml organ bath containing Tyrode’s solution of the following composition: NaCl, 136.6; KCl, 2.7; CaCl₂, 1.8; NaHCO₃, 12.0; MgCl₂, 0.5; Na₂PO₄, 0.14 and glucose, 5.5. The set up was maintained at 37±1 °C and aerated with 95% O₂ and 5% CO₂. A load of 1.0 g was applied and a 60 min equilibration period was allowed, during which the physiological solution was changed every 15 min. At the end of the equilibration period, the effects of serotonin (1.74×10⁻⁵ to 5.5×10⁻⁵ M) and methyl angolensate (1.06×10⁻⁴ to 6.8×10⁻³ M) were evaluated. The blocking effect of methyl angolensate (8×10⁻⁴ to 1.7×10⁻³ M) and cyproheptadine (1.5×10⁻⁸ M) were investigated on serotonin-induced contractions of the fundus strip. Responses were recorded on a Ugo Basile Unirecorder 7050 via an isometric transducer. Determinations were done

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Studies on Guinea Pig Ileum  Guinea pig of either sex were starved overnight but had free access to water. The animals were killed by a blow on the head, exsanguinated and the abdomen opened. The ileum was removed and cut into segments 2 to 3 cm long. The segments were dissected free of adhering mesentery and mounted in a 20 ml organ bath containing Tyrode’s solution gassed with 5% CO₂ and 95% O₂ and maintained at 37±1 °C. A load of 0.5 g was applied. The tissue was equilibrated for 60 min during which the bathing solution was replaced every 15 min. At the end of the equilibration period, the inhibitory effects of methyl angolensate (8×10⁻⁴ to 1.7×10⁻³ M) and mepyramine (2.5×10⁻⁶ M) on histamine (4.5×10⁻⁷ to 7.2×10⁻⁶ M)-induced contractions of the guinea pig ileum were evaluated. Responses were recorded on a Ugo Basile Unirecorder 7050 via an isometric transducer 7004.

Studies on the Rabbit Jejunum  Adult rabbits of either sex were killed by a blow on the head, exsanguinated and the abdomen opened. Segments of the jejunum about 2—3 cm long were removed and dissected free of adhering mesentery. The tissue was mounted in a 20 ml organ bath containing Tyrode’s solution at 37±1 °C and aerated with air. A load of 0.5 g was applied. A 60 min equilibration was allowed during which the physiological salt solution was changed every 15 min. The inhibitory effect of methyl angolensate (1.06×10⁻⁴ to 6.8×10⁻³ M) was evaluated on spontaneous contractions of the rabbit jejunum. The inhibitory effects of methyl angolensate (8×10⁻⁴ to 1.7×10⁻³ M) and atropine (5×10⁻⁹ M) on acetylcholine (2.5×10⁻⁷ to 1.0×10⁻⁷ M)-induced contraction of the rabbit jejunum were also evaluated. Responses were recorded on a Ugo Basile Unirecorder 7050 via an isometric transducer. Determinations were done in quadruplicate.

Studies on Gastrointestinal Motility in Mice  The method of Capasso et al. was adopted to test the effects of methyl angolensate on gastrointestinal motility in mice. Adult Swiss albino mice were starved for 24 h prior to the experiment, but were allowed free access to water. One group was given 20 ml/kg of normal saline, while three groups received methyl angolensate at doses of 200, 400 and 800 mg/kg p.o., respectively. The last group received atropine (0.1 mg/kg, i.p.). Five minutes after drug administration, 0.5 ml of a 5% charcoal suspension in 10% aqueous solution of tragacanth powder was administered to each animal orally. The animals were sacrificed 30 min later and the abdomen opened. The percentage distance of the small intestine (from pylorus to caecum) travelled by the charcoal plug in both treated and normal saline groups were determined.

Statistical Analysis  Results were expressed as mean±S.E.M. Statistical analysis of the data was done using Analysis of variance and Bonferroni test. The results were considered significant when p<0.05.

RESULTS  Effect of Methyl Angolensate on the Rat Stomach Fundus  Methyl angolensate (1.06×10⁻⁴ to 6.8×10⁻³ M) was found to cause a concentration dependent relaxation of the fundus strip. Pre-incubation of the tissue with methyl angolensate (8×10⁻⁴ to 1.7×10⁻³ M) was found to inhibit serotonin (1.74×10⁻⁵ to 5.5×10⁻⁵ M)-induced contractions of the fundus strip dose-dependently. Cyproheptadine (1.5×10⁻⁸ M)
also inhibited the contractions evoked by serotonin (Fig. 2).

Effect of Methyl Angolensate on Guinea Pig Ileum
Methyl angolensate (1.06×10⁻⁴ to 6.8×10⁻³ M) did not cause any remarkable effect on the isolated guinea pig ileum. Histamine (4.5×10⁻⁷ to 7.2×10⁻⁶ M) caused a concentration dependent contraction in this preparation. Methyl angolensate at concentrations of 8×10⁻⁴ to 1.7×10⁻³ M did not affect the responses caused by histamine. Mepyramine (2.5×10⁻⁶ M) inhibited the contractions induced by histamine (Fig. 3).

Effect of Methyl Angolensate on the Rabbit Jejunum
Methyl angolensate (1.06×10⁻⁴ to 6.8×10⁻³ M) exhibited a concentration dependent inhibition of the spontaneous contractions of the rabbit jejunum (Fig. 4). The relaxation shown by methyl angolensate did not attenuate acetylcholine (2.5×10⁻⁹ to 1×10⁻⁷ M) induced contractions of the isolated rabbit jejunum, but atropine (5×10⁻⁹ M) did (Fig. 5).

Effect of Methyl Angolensate on Gastrointestinal Transit
Methyl angolensate (200, 400 and 800 mg/kg, p.o.) reduced significantly (p<0.05) small intestinal transit in a dose related manner. Atropine (0.1 mg/kg, i.p.) caused about 49.54% percent inhibition of the charcoal transit in mice (Table 1).

DISCUSSION
This study has provided preliminary data on the effect of
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methyl angolensate, a triterpenoid isolated from *Entan-

dropha magma angolense*, on non-vascular smooth muscles. Methyl angolensate produced a concentration dependent in-
hibition of the rat fundus strip, rabbit jejunum and was de-

![Methyl angolensate (mg/ml)](image)

**Fig 5. Concentration-Dependent Relaxation of the Rabbit Jejunum Caused by Methyl Angolensate (0.05—1.6 mg/ml)**

**Table 1. Effect of Methyl Angolensate (MA) on Gastrointestinal Transit in Mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Distance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>30 ml/kg</td>
<td>86.7±7.3</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.10</td>
<td>43.8±5.7*</td>
</tr>
<tr>
<td>MA</td>
<td>200</td>
<td>47.0±11.1*</td>
</tr>
<tr>
<td>MA</td>
<td>400</td>
<td>28.9±4.7*</td>
</tr>
<tr>
<td>MA</td>
<td>800</td>
<td>19.6±4.0*</td>
</tr>
</tbody>
</table>

* Significantly different (*p*<0.05) from control.

is also known that 5-HT released by mechanical or vagal stimulation also acts locally to regulate gastrointestinal func-
tions. Motility of gastric and intestinal smooth muscles may be enhanced or inhibited via 5-HT receptors.17) Methyl an-

golensate was found to inhibit serotonin-induced contrac-
tions of the rat fundus, suggesting that the effect of methyl angolensate on the gastrointestinal smooth muscles could be medi-
ated through inhibition of 5-HT$_{2B}$ serotonergic receptors. The charcoal meal test, allows for comparative evaluation of the
degree of inhibition or stimulation of gastrointestinal mo-
bility in laboratory animals.15) Our findings showed that methyl angolensate reduced gastrointestinal transit in mice agreeing with some of the results of *in vitro* studies in which the extract relaxed the gastrointestinal smooth muscles. It is
therefore possible that these properties of methyl angolensate may contribute to the overall effects of the plant in humans. We may conclude therefore that methyl angolensate, a com-
ponent of *E. angolense* plant, might contribute positively to the traditional use the plant against ulcer, possibly by form-
ings a protective coat on the gastrointestinal tract and by inhi-
bition of 5-HT$_{2B}$ receptors in rat fundus.

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