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Methyl Angolensate: The Antiulcer Agent of the Stem Bark of *Entandrophragma angolense*

Vincent C. O. Njar[1,3], *Julius K. Adesanwo*[1], *and Yinusa Raji*[2]

[1] Department of Chemistry, University of Ibadan, Ibadan, Nigeria
[2] Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[3] Address for correspondence

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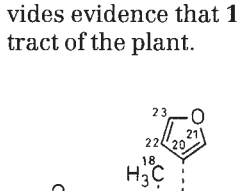
Abstract

Methyl angolensate (**1**), the major compound isolated from the methanol extract of the stem bark of *Entandrophragma angolense* produced a dose-related inhibition of gastric ulceration, 40 mg/kg body weight (B. W.) being more effective than 40 mg/kg B. W. of propranolol. The highest dose used (80 mg/kg B.W.) completely inhibited gastric ulceration and significantly reduced gastric acidity ($P < 0.05$). Furthermore, **1** (40 mg/kg B. W.) significantly reduced gastric acid secretion induced by histamine (1.0 mg/kg B. W.) and carbachol (1.0 mg/kg B. W.). These results suggest that **1** produces its antiulcer activity by inhibition of gastric acid secretion.

We have recently shown (1) that the methanol extract of the stem bark of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae) is highly potent in inhibiting indomethacin-induced gastric ulceration in rats. We also showed that the crude extract was non-toxic to the rats at a wide range of doses (20–200 g/kg B. W.) tested. These observations prompted us to undertake a phytochemical study of the plant aimed at isolating and identifying the antiulcer agent(s). This paper describes the isolation and identification of methyl angolensate (**1**) from the methanol extract of the stem bark of *E. angolense* and provides evidence that **1** is the antiulcer agent of the crude extract of the plant.



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Fractionation of the methanol extract of the stem bark of *E. angolense* led to the isolation of **1** as the major compound. The identity of **1** was established by spectroscopy (UV, IR, OR, ^{1}H-NMR, EI-mass) and by comparison of our data with those reported in the literature (2–4). Consequently, the efficacy of **1** to protect against experimental indomethacin-induced gastric mucosal damage and to influence gastric acid secretion was investigated as indicated in the Materials and Methods section. The results presented in Table **1** clearly show that **1** produces a dose-related gastroprotective action in indomethacin-induced ulceration in rats. The cytoprotection produced by propranolol (40 mg/kg) was lower than that caused by 40 mg/kg of **1**. Thus, **1** is more potent than propranolol in protecting against gastric ulceration in rats. These results are identical to those that we have previously reported (2) for the methanol extract of *E. angolense* and they confirm that **1** is the antiulcer agent of the stem bark of *E. angolense*.

The significant reduction in total intragastric acidity observed in this study (see Table **1**) strongly suggests that **1** may act by inhibiting gastric acid secretion by the parietal cells. To investigate the mechanism of ulcer inhibition, the effects of **1** (40 mg/kg B. W.) on basal, histamine- (1 mg/kg, B. W.), and carbachol- (1 mg/kg, B. W.) induced gastric acid secretion in male albino rats were studied. The results (mean of 5 experiments ± SEM) are as

Planta Med. 61 (1995) 91–92

Treatment[a]	Ulcer Index[b]	Inhibition of ulceration[c] (%)	Total gastric acid content (μEq HCl/100 g B.W.)
Control (2% Tween 80, 4 ml/kg B. W.)	2.83 ± 0.62	–	59.7 ± 13.9
Methyl angolensate (**1**, mg/kg B. W.)			
20	1.55 ± 0.06[d]	45.3	36.0 ± 5.7
40	0.60 ± 0.07[d]	78.8	19.7 ± 5.1
80	0.00 ± 0.00[d]	100.0	18.1 ± 8.5
Propranolol (40 mg/kg B. W.)	1.17 ± 0.62	58.7	43.8 ± 9.2

Table 1 Effect of methyl angolensate (**1**) on gastric mucosal lesion and total acid content induced by indomethacin.

[a] Five animals were used in each test.

[b] $\text{Ulcer Index} = \frac{\text{Mean degree of ulceration} \times \% \text{ of group ulceration}}{100}$.

[c] $\text{Inhibition of ulceration} = \frac{\text{Ulcer index in control} - \text{Ulcer index in test}}{\text{Ulcer index in control}} \times 100$.

[d] Significant compared with control ($P < 0.05$).

follows: (i) Effect of **1** and histamine (basal, 0.2 ± 0.01; **1**, 0.08 ± 0.01, histamine, 0.83 ± 0.02). (ii) Combined effect of **1** and histamine (basal, 0.32 ± 0.02; **1**, 0.26 ± 0.01; histamine, 1.20 ± 0.02; **1** plus histamine, 0.54 ± 0.02). (iii) Combined effect of **1** and carbachol (basal, 0.18 ± 0.01; **1**, 0.13 ± 0.01; carbachol, 0.96 ± 0.01; **1** plus carbachol, 0.58 ± 0.02). They clearly show that **1** reduced basal, histamine-, and carbachol-induced gastric acid secretion, indicating that the compound competes with histamine receptors as well as blocking the cholinergic mechanism of gastric acid secretion. Because of the high antiulcer activity exhibited by **1**, further experiments aimed at developing a new class of antiulcer drugs may be interesting.

Materials and Methods

The respective spectra were recorded with the following instruments: IR, Analect 6260 FX FTIR; UV, Varian DMS-100 UV/VIS; Optical rotation, Perkin-Elmer 241; EIMS, Kratos Aspect System. ^{1}H-NMR, Brucker AC 400 at 400 MHz.

Animals

Male albino Sprague-Dawley rats (150–180 g) were obtained from the Animal House, College of Medicine, University of Ibadan, Ibadan, Nigeria. They were maintained under standard laboratory conditions and were fed normal rat chow and tap water *ad libidum*.

Plant material, extraction, and fractionation

The stem bark of *E. angolense* was collected from the Forest Reservation at Ijebu-Ode, Nigeria. The plant was identified by Dr. Joyce Lowe of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria.

The air-dried pulverized stem bark (200 g) was extracted as previously described (1) to give a powdery crude extract (15 g). 5 g of the extract were chromatographed on a silica gel column (2.5 × 48 cm), eluting with hexane/EtOAc mixtures of increasing polarity (5% EtOAc/hexane (400 ml), 15% EtOAc/hexane (400 ml), 30% EtOAc/hexane (200 ml), 40% EtOAc/hexane (300 ml), and 50% EtOAc/hexane (200 ml). The fractions eluted with 40% EtOAc/hexane were similar, so they were combined and concentrated to give a dirty white solid (1.73 g). Crystallization from hexane/EtOAc gave a crystalline white solid (**1**, 1.15 g, 2.27% yield); m.p. 198 °C; $[\alpha]_D^{20}$: −43.3°. Satisfactory spectral data were obtained for **1** which agree with those reported in the literature for methyl angolensate (2–4). Copies of the original spectra are obtainable from the author of correspondence.

Pharmacological test

Gastric lesions experiments were performed as previously described (1) according to the methods of Elegbe (5) and Zaidi and Mukerji (6). The total gastric acidity of rat gastric contents was determined according to the method of Lai (7). Gastric acid secretion responses to **1**, histamine, and carbachol were carried according to the method of Ghosh (8).

Statistical analysis

Statistical analysis was performed using Student's t-test and significance of difference was accepted at $p < 0.05$. Data are presented as mean ± SEM.

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