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Three pyridinium alkaloids may account for the antibiotic effect of the seed of *Abrus precatorius*

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ABSTRACT

A methanol extract of Abrus precatorius seed cotyledons has been found to exhibit growth inhibitory activity against both Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). This activity was traceable to three pyridinium components viz: - trigonelline, precatorine and a sugar ester of trigonelline. These alkaloids may contribute to the use of the seed extracts in folk medicines for the treatment of infective disorders.

Keywords: *Staphylococcus aureus*, *Escherichia coli*, trigonelline, precatorine.

INTRODUCTION

Preparations from the seeds of *Abrus precatorius* Lin. (Fabaceae or Leguminosae) are used, with varying degrees of success, in folk medicines as a remedy for various infections. In parts of Eastern Nigeria, aqueous decoctions of the seeds are used to treat cough, ulcer, diarrhea, dysentery and venereal diseases. A poultice of the seeds is also used in India for similar purposes and for skin infections [1, 2]. Infusions of the seeds are considered to be of value in eye infections in Eritrea [3, 4], Brazil [5] and Central Africa [6] especially Tanzania [7].

[8] in 1996 then [1] in 2007 have reported antibiotic activity for alcohol extracts of the whole seeds of which they ascribed to gallic acid and another unidentified phenolic component, both present in the seed testa. The cotyledons per se have not so far been studied for antibiotic potential, which is the subject of this examination. This is necessitated by antibiotic resistance that is now a global concern. It is also predicated on the emergence of new diseases and the failure of or expensiveness of orthodox drugs.

MATERIALS AND METHODS

Plant materials: *Abrus* seeds were collected from Ukehe, Enugu State, Nigeria and were authenticated at source by Dr. C.O.C Agwu of our Botany Department. A voucher specimen is retained in this laboratory.

Pulverized decorticated *Abrus* seed cotyledons were macerated twice in 5-volume (w:v) chloroform-methanol (2:1) for 15h. The macerate was decanted through Whatmant No. 1 filter paper and the filtrate was shaken with 0.2- volume water. The two layers obtained were separated and then evaporated to dryness under reduced pressure. The upper aqueous methanol layer was subjected to thin layer chromatography (TLC) on silica gel $F_{254/366}$, using n-butanol-acetic acid-water (65:13:22). Five bands, A-E of Rf values 0.1, 0.24, 0.57, 0.71 and 0.78, respectively were obtained. Band A was recovered and further fractionated into three components A1-A3 (Rf values: 0.1, 0.19 and 0.35, respectively) by TLC on the same grade of silica gel using methanol-chloroform (9:1).

Detection of the bands was by visualization under short wave UV light and by reactions with chromogenic Dragendorf's reagent for alkaloids. Further evidence from spectroscopy and the comparison of Rf data with those of authentic specimens were used to confirm the identity of these fraction as trigonelline, precatorine and a dibasic sugar alkaloid containing a trigonelline moiety as follows below.

Ultra violet (UV) spectra of methanol solutions of A1-A3 were obtained on a Pye Unicam Cambridge SP 1800 spectrophotometer. Proton –NMR spectra were obtained on a 200 MHz Nicolet instrument, in D_2O solution with TMS as internal standard and EIMS was at 70 eV on a Micromass mass-spectrometer.

A3: trigonelline. Uv max (nm): 216, 265, 285sh. ^{1}H-NMR (D_2O) ppm: 9.14 (1H, s); 8.62-8.60 (2H, d); 8.40-8.24 (1H, t); 4.51 (3H, s). MS, m/e (%): 138 M^+, 80.6); 123 (11.3); 94 (42.7); 44 (100). All the data above were identical to those obtained for an authentic sample (Sigma) of trigonelline. Co-tlc with the authentic sample further confirmed the identity.

A2: precatorine. UV max (nm): 216, 265, 290sh. ^{1}H-NMR (D_2O) ppm: 9.14 (1H,s); 8.66-8.60 (2H, d); 8.40-8.24 (1H, t); 7.10-7.05 (H,s); 4.45 (3H, s); Ms: m/e (%) 290 (M^+, 7.0); 169 (gallate, 11.0); 153 (20.0); 137 (trigonelline, 17.3); 126 (16.3); 123 (20.4); 94 (30.2); 78 (7.33); 57 (100); 44 (11.3). Acid hydrolysis (0.1M H_2SO_4 X 1h boiling) afforded gallic acid and trigonelline (identification by comparison of uv, ms, ^{1}H-nmr and co-tlc with authentic sample).

A1: sugar ester of trigonelline .UV max. nm; 216, 265, 272sh ^{1}H-nmr (D_2O) ppm: 9.24 (1H, s);8.94-8.90 (2H,s); 8.12-8.05 (3H, t);4.47 (3H,s); 4.25-1.75 (unresolved signals.) Ms: m/e (%) 469 (M^+, 16.5); 412 (10.7); 372 (11.2); 218 (19.2); 203 (44.2); 167 (9.9); 163 (16.3); 94 (12.4); 83 (30.5); 71 (26.5) 57 (52.9); 44 (100); 43 (90.4). acid hydrolysis (2M H_2SO_4 x 2h boiling) yielded trigonelline, a sugar and another alkaloid (both not identified).

Antibacterial activity determinations:
*Staphylococcus aureus (*ATCC 12600) and Eschericia Coli (ATCC 11775) (both from our Microbiology Department) were used. The filter paper disc method was used to assess sensitivity based on zones of inhibition. Minimum inhibition concentrations (MIC), using constant inocula (10^8 cell / ml) in nutrient broth containing scalar concentrations of seed extracts A1- A3 were determined spectrophotometrically at 540 nm. Triplicate determinations were done all incubations were at 37^0 C for 18 h.

RESULT AND DISCUSSION

As shown in table 1, lincocin (standard antibiotic), the methanol fraction, M, and three of its alkaloidal fractions viz A1, A2, and A3 were effective against the growth of both *S. Aureus and E. coli*. This finding correlates the observation [8, 2] that the alcohol extracts of *Abrus* seeds exhibit antibacterial activity. The data presented show that this activity is due to the presence of at least three antibiotic substances A1, A2 and A3.

By direct comparison of chromatographic and spectroscopic characteristics, A3 was found to be trigonelline. Similarly, the spectroscopic data of A2 and the presence of gallic acid and trigonelline in its acid hydrolysate agree with literature data for precatorine [9]. Both alkaloids were previously demonstrated in the seeds by [9] and [10]. For A1, the three-proton singlet at 4.47 and the four aromatic signals in its proton-nmr spectrum confirm a molecular species containing trigonelline [9]. From the fragmentation pattern of authentic trigonelline samples, the fragment ions at m/e 137, 123, 94, 44 are diagnostic of trigonellinyl moiety. The identification of this moiety in the acid hydrolysate of A1 supports the conclusion that A1 IS A trigonellinyl compound. Both A1 and one component of its acid hydrolysate reacted positively to α-naphthol and Fehling's solution showing that A1 contains a carbohydrate moiety with free carbonyl function.

This investigation revealed that the cotyledons of Abrus seeds contain three pyridinium antibiotic substances, which together with the phenolic reported by [8] and [2], account for the use of the seeds in folk medicine for the treatment of infective disorders. It is however, not certain whether this unidentified phenolic is indeed precatorine, the gallic acid ester of trigonelline. A sweet tasting saponin substance present in the seeds [11] may after all be the culprit.

Table 1: Growth inhibitory activity on *S. aureus* and *E. coli*

Test Substance	Zone of Inhibition (mm)		Mic (µg/ml)	
	S. aureus	***E. coli***	***S. aureus***	***E. coli***
M	28	21	-	-
A1	32	28	10	20
A2	27	28	30	50
A3	21	18	50	100
Lincocin	30	22	12.5	25

The greatest sensitivity of S. aureus to the pyridinium alkaloids is in consonance with, and extends earlier observation [12] that quaternary nitrogen antibiotics are more active against gram- positive bacteria. Protein binding capacity was indeed demonstrated recently for these three quaternary alkaloids in this laboratory (unpublished observations). Like many quaternary

nitrogen heterocycles, they may exert their effects by electrophilic attack of the imminium ion on biological nucleophiles such as DNA and membrane protein.

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