

## ISOLATION OF N,N-DIMETHYL-N-(2-OXO-1,2-DIHYDROPYRIDIN-3-YL)IMIDOMETHANAMIDE FROM METHANOL EXTRACT OF THE VARIETY MINOR SEEDS OF *RICINUS COMMUNIS* LINN. (*EUPHORBIACEAE*)

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### ABSTRACT

White crystals, M3, have been isolated from the methanol extract of the smaller *Ricinus communis* seed variety. The melting point of M3 was found to be 206-208°C and its TLC profiles using four different solvent systems (I-100% ethyl acetate, II- chloroform : acetone = 3:2, III- chloroform : acetone = 1:2 and IV- 100% acetone) indicated single spots with respective  $R_f$  values of 0.14, 0.54, 0.66 and 0.72. Phytochemical test, using Dragendorff's spray reagent, of M3 gave positive test for alkaloid. The Tandem MS/MS spectrum of M3 confirmed the structure to be N,N-dimethyl-N-(2-oxo-1,2-dihydropyridin-3-yl)imidomethanamide. The infra-red (IR) spectrum of M3 provided absorption bands in conformity with the structure of this alkaloid. To the best of our literature search, this is the first report for the isolation of this alkaloid from the *Ricinus communis* plant.

**KEY WORDS:** *Ricinus communis*, variety minor seeds, methanol extract, alkaloid

### INTRODUCTION

*Ricinus communis* belongs to the Euphorbiaceae family, a diverse and economically important family of flowering plants. The plant has many common names such as castor plant, castor oil plant, castor bean plant, wonderboom, dhatura, eranda, Palma Christi and so on. Locally the plant is known in Nigeria by such names as "Zurman" (Hausa), "Laraa" (Yoruba), "Ogilisi" (Igbo), "Kpamfini gulu" (Nupe), "Jongo" (Tiv), and "Era ogi" (Bini). The castor plant is considered by most authorities to be native of the Tropical Africa, and may have originated in Abyssinia (Weiss, 1971). The plant is said to be a native of India (Trease and Evans, 1985) with about 17 species which have been grouped into shrubs and trees that produce large seeds and a second group of annual herbs that produce smaller seeds. Attempts have been made to classify varieties of the plant based on the seed size and colour, growth habit, and foliage colour. The seeds of the castor plants which grow in the northern states of Nigeria have been classified into seven distinct varieties according to their sizes and colours (van Rheenen, 1976). Researchers (Das *et al.*, 2000) however, have always classified the seeds into three groups depending on the seed size and include the large size seeds (variety major), medium size seeds (variety intermediate) and the small size seeds (variety minor).

Tanira and co-workers reported the absence of alkaloids, coumarines, and volatile oils was reported in the entire plant (Tanira *et al.*, 1989), however, the same workers have found the presence of flavonoids, anthraquinones, saponins, tannins, sterols and/or triterpenes in the plant. El-Tawil had previously reported the presence of alkaloids and coumarines in the plant but but flavonoids, saponins, sterols and/or

triterpenes were found to be absent (El-Tawil, 1983). Alkaloids have also been reported to be present in the leaves and seeds (Coe and Anderson, 1996). The pyrrolizidine alkaloids have already been reported to be present in the seeds (Arseculeratne *et al.*, 1985). More recently, the seeds (variety minor) were reported to contain steroids and alkaloids but triterpenes, flavonoids and tannins were not detected (Kabele-Toge *et al.*, 1996).

The alkaloid indole-3-acetic acid was reported to have been isolated from the roots of the plant (Hall and Medlow, 1974). It was also reported that the alkaloids ricinine and N-demethylricinine have been isolated from the leaves (Khafagy *et al.*, 1983; Shukla *et al.*, 1992). Ricinine was also isolated from the flowers (Khafagy *et al.*, 1979) and from the seeds (Pahuja *et al.*, 1979; Bukhatchenko, 1980; Borodulina and Bukhatchenko, 1980; Hilal *et al.*, 1982; Gao *et al.*, 2000).

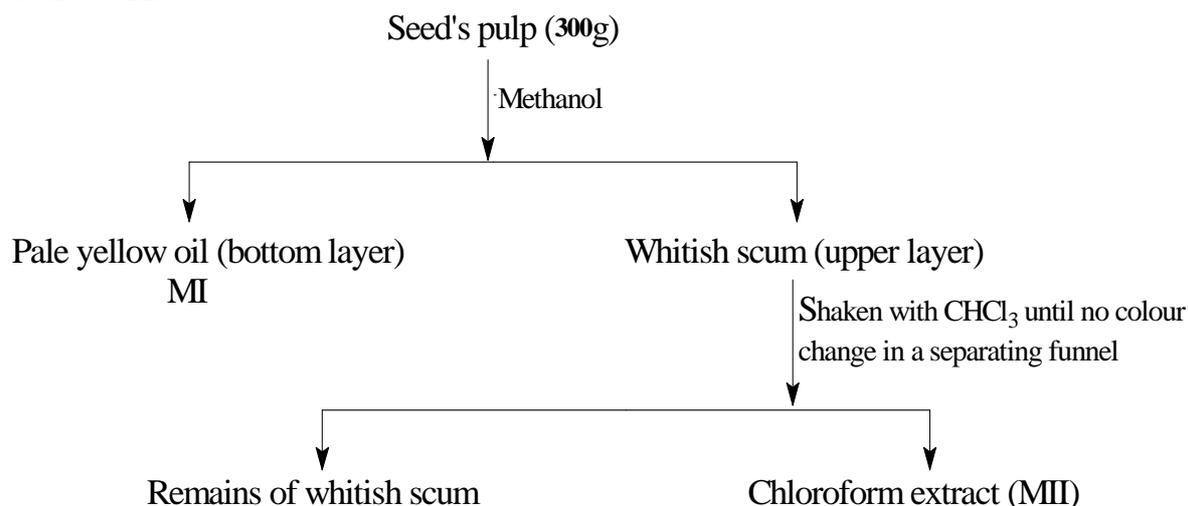
### MATERIALS AND METHODS

#### *Plant Material*

The seeds (variety minor) of *Ricinus communis* were collected from Kano between Kofar Dan'agundi and Kofar Na'isa along Bayero University Road. The seeds were authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a herbarium specimen was deposited (Voucher Number: 900233).

#### *Methanol Extraction*

The wet ground pulp (300g) was extracted with Methanol using a magnetic stirrer for 1 hour. The weight and percentage of the pale yellow oily extract (MI) was recorded.



The chloroform extract of the whitish scum of the RC1 was collected and coded MII. Greenish-white solids deposited in this extract after 15 days and these were filtered and washed with petroleum ether. The washed solid was coded M2 and the petroleum ether wash as MII-1. White solids were seen suspended in MII-1 after being left standing for two weeks and these were filtered, washed with chloroform, re-crystallized from methanol and coded as M3. The TLC picture and melting point of M3 have been recorded. The M3 was studied using IR spectrometry and Tandem MS/MS Spectrometry. The IR spectrum was recorded with KBr disc using attenuated total reflectance (ATR) on a Golden Gate Diamond ATR Spectrometer (Perkin-Elmer Spectrum One Model). The Tandem MS/MS Spectrometry was recorded on ThermoFinnigan LCQ™ Classic, coupled with Fast Atom Bombardment (FAB) ionization technique.

#### Phytochemical Screening

The presence of alkaloids in the extracts were investigated using the methods of Trease and Evans (1983). The purified crystals were also tested for alkaloids using Dragendorff's spray reagent on silica-gel coated plates.

#### RESULTS

##### *Thin layer chromatography and melting point of M3*

The melting point of the white crystal, M3, was found to be 206-208°C and its TLC profiles using four different solvent systems (I - 100% ethyl acetate, II - chloroform : acetone = 3:2, III - chloroform : acetone = 1:2 and IV - 100%

acetone) indicated single spots with their respective  $R_f$  values of 0.14, 0.54, 0.66 and 0.72.

#### Phytochemical Screening

Phytochemical test of M3 gave positive test for alkaloid. This crystal, when spotted on TLC plate, sprayed with the Dragendorff's reagent and activated in an oven at 100°C for 1 hour, produced an orange-brown spot.

#### Infra-red (IR) Spectroscopy

The IR spectrum of M3 is shown in Figure 1 and the identified diagnostic absorption bands for the alkaloid are as follows. The typical diagnostic band for nitrile  $C\equiv N$  (2224  $cm^{-1}$ ) is missing, indicating the absence of this functional group on the 2-pyridone ring. The other prominent peaks are seen at 2852.76  $cm^{-1}$  (N-CH<sub>3</sub>, aliphatic methylamino), 2958.50  $cm^{-1}$  (=C-H aliphatic stret.), 3038  $cm^{-1}$  and 3104  $cm^{-1}$  (=C-H aromatic stret.), 1495.79  $cm^{-1}$ , 1536.81  $cm^{-1}$  and 1603.16  $cm^{-1}$  (C=C in-plane aromatic skeletal vibrations, stret.), 1636.64  $cm^{-1}$  (C=O, 6-membered lactam, stret.) and 3408.78  $cm^{-1}$  (N-H, secondary amide, stret.).

#### Tandem MS/Mass Spectrometry

The sample, M3, was ionized in the ion source of MS-1 using fast atom bombardment as the "soft" ionization technique. The molecular ion spectrum is provided in Figure 2. The beam of the single molecular ion (with peak at  $m/z$  165) was further fragmented by colliding with helium gas in a collision cell.

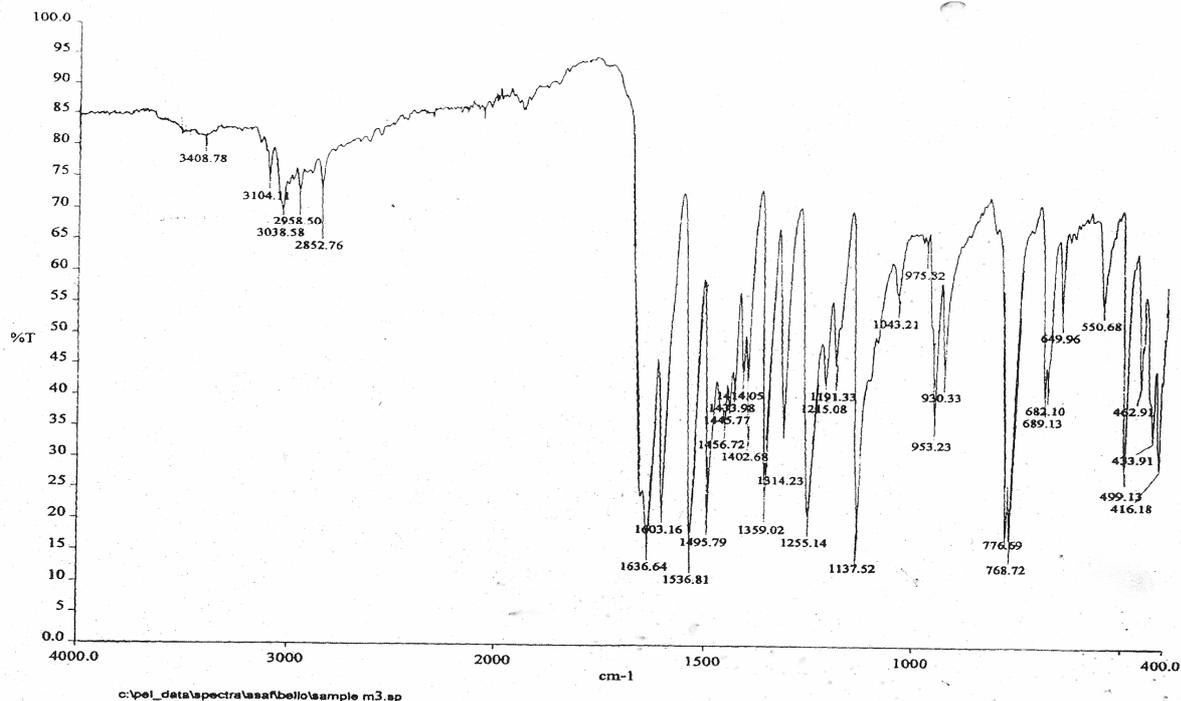


Figure 1: Infra-red (IR) spectrum of M3

D:\0307052  
M3

03/07/2005 09:43:15 AM

standard source, pos ion

0307052 #771-810 RT: 11.43-11.86 AV: 40 NL: 1.18E7  
T: + c Full ms [ 50.00-500.00]

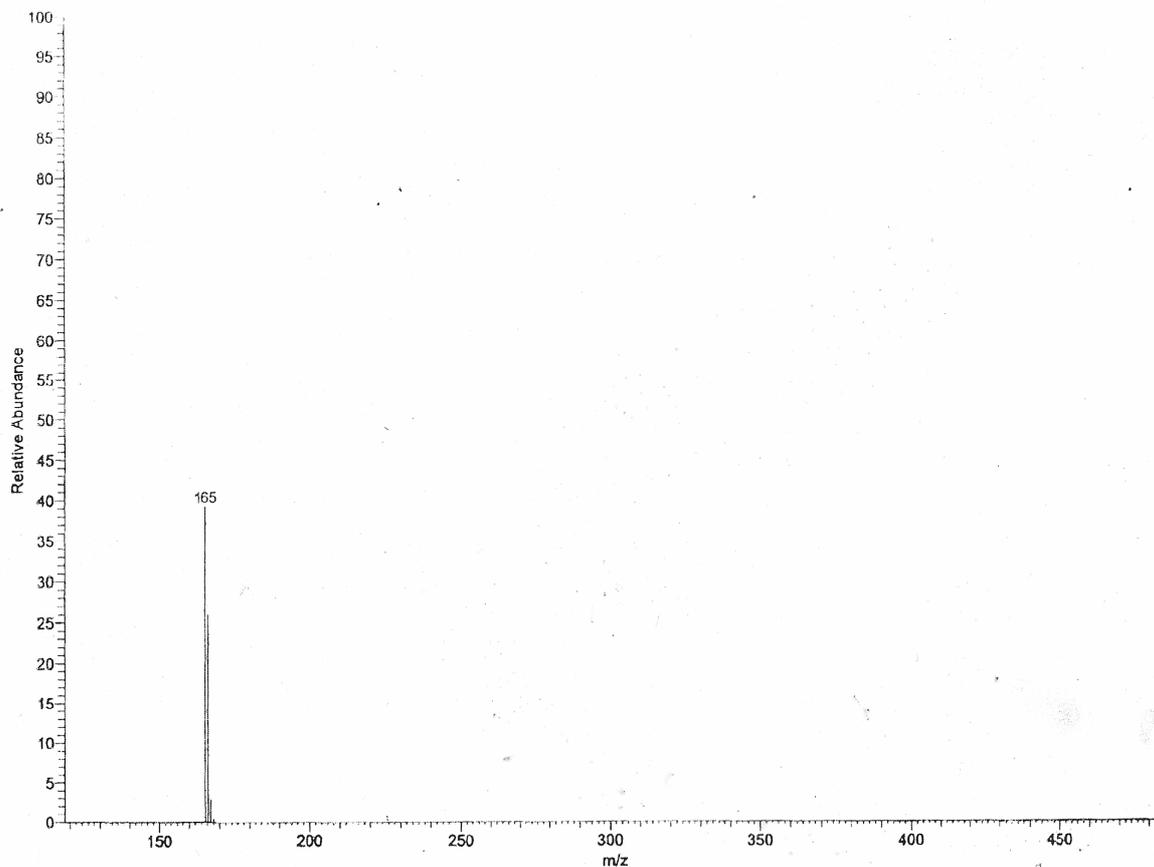


Figure 2: Molecular ion spectrum of M3

The resulting fragment ions were mass analyzed in MS-2 to afford the mass spectrum given in Figure 3.

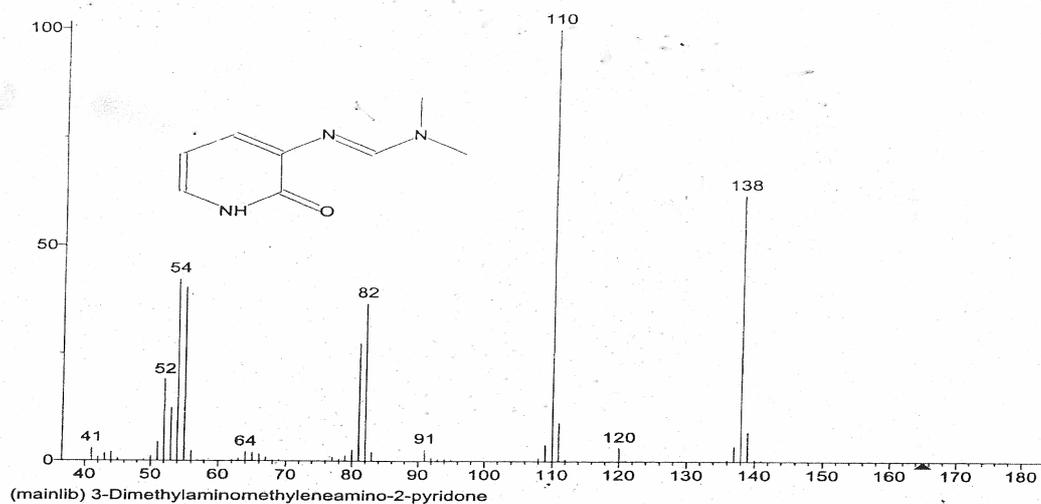
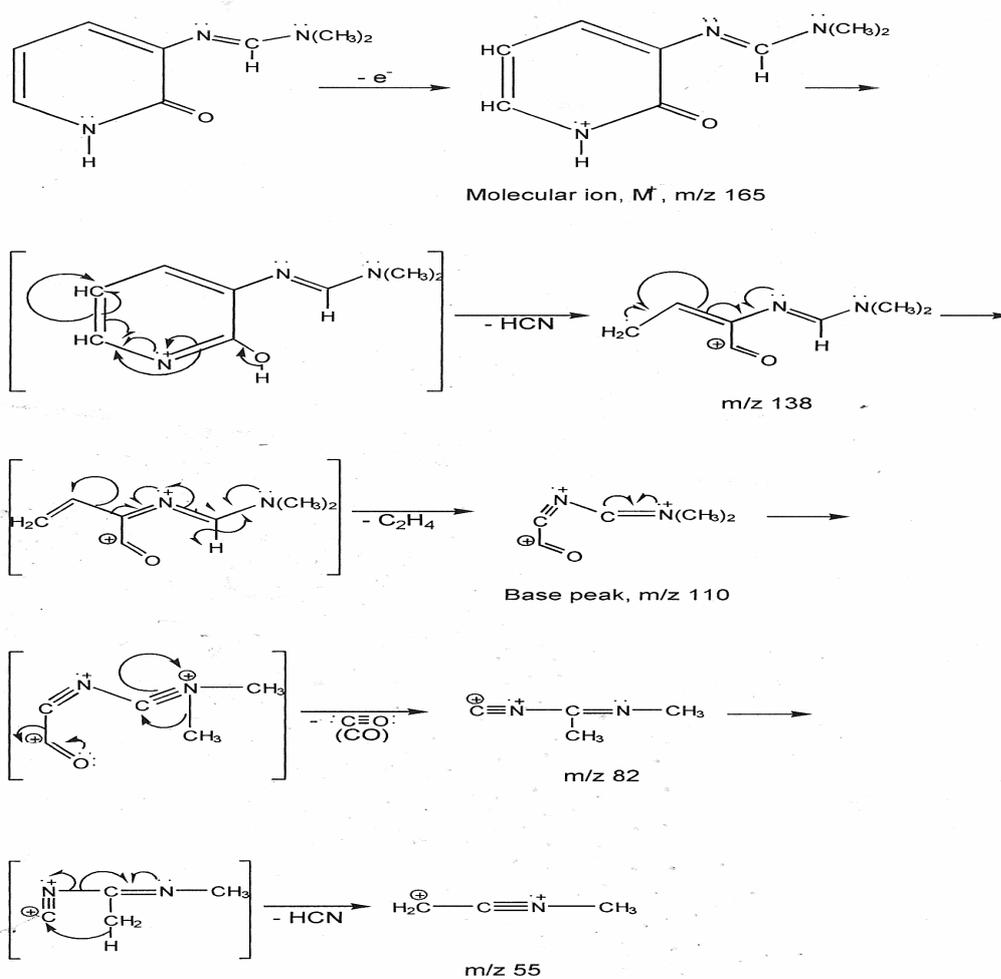


Figure 3: Computer search provided matched mass spectrum of M3  
 The fragmentation pattern of the compound is provided in scheme 1.0.



Scheme 1: Molecular mass fragmentation pattern of M3

## DISCUSSION

The IR spectrum of M3 provided absorption bands in conformity with the structure of this alkaloid. The Tandem MS/MS spectrum of the white crystals (M3) was computer searched and found to match very well with that of 3-dimethylaminomethyleneamino-2-pyridone. The systematic name of this compound is *N,N*-dimethyl-*N'*-(2-oxo-1,2-dihydropyridin-3-yl)imidomethanamide or *N,N*-dimethyl-*N'*-(2-oxo-1H-pyridin-3-yl)methanimidamide. Many alkaloids have been reported (Trifunac and Bernstein, 1982) to inhibit the metabolism and motility of human spermatozoa. Generally, the 2-pyridone ring (contained in the alkaloid, M3) is considered a novel ring and its derivatives, natural and synthetic, have several pharmacological activities including antibacterial, antiviral and antitumor activities (Qun *et al.*, 2000; Torres *et al.*, 2005). Alkaloids, containing this ring, which have various CNS activities were isolated from the extracts of the fungi *Paecilomyces militaris*

(Khachatourians, 1996), and biogenetically related pyridone antifungals have been isolated from other fungal genera such as *Beauveria amorphia* (Khachatourians, 1996). Some 2-pyridone derivatives have also been found to have dopaminergic activities as they demonstrated inhibitory effects on prolactin secretion from the anterior pituitary in rats (Yoon *et al.*, 1993). The synthesis of a substituted 2-pyridone ring is now rather a topical area of continuing interest due to the number of biologically active molecules containing this moiety. Many naturally occurring and synthetic derivatives of 2-pyridone are patented due to their biological activities including the CNS activity. Long ago, this alkaloid (M3) plus eight others were isolated from twelve species of the plant *Aspidosperma dasycarpon* A. DC. (Joule *et al.*, 1965) and since then the alkaloid has been synthesized and patented. However, this is the first report of the isolation of this alkaloid from the *Ricinus communis* plant.

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