

# Constituents of the ethanolic extract of *Bryophyllum Pinnatum* leaves

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## Abstract.

*Bryophyllum Pinnatum* commonly called cant bury bells of the family crassulaceae is a perennial herb. Soxhlet extraction of the fresh leaves with ethanol and chromatography of the crude gave two compounds – a pungent oil (1)  $R_f$  0.75 identified as 4-ethoxy-cyclopent-2-enone and another oil (3)  $R_f$  0.52 also identified as 2,5-dimethoxyfuran by their spectra data such as infra red, mass and proton magnetic resonance spectra. The structure of (3) was further confirmed by synthesis by the oxidation of furan(2) with bromine in methanol and comparing the spectra of the synthetic compound with those of the naturally occurring compound. A significant feature of (3) is that it is a mixture of two isomers with H-2 and H-5 protons appearing as singlets at  $\delta$ 6.00 and 5.91. The two methoxy groups also appeared as two singlets at  $\delta$ 3.32 and 3.28.

**Keywords:** *Bryophyllum Pinnatum*, soxhlet extraction, 4-ethoxy-cyclopent-2-enone and 2,5-dimethoxyfuran

## I. INTRODUCTION

The heavens, earth and all things therein including plants were created by God. He gave man the authority to have dominion over the earth, hence man began to use plants for food, clothing, shelter, fire and medicine (Nsi and Dyegh, 2004). The phytochemicals present in plants are the ones utilised in various ways such as in the treatment and control of diseases (Ayandele and Adebisi, 2007). In other cases, some plants have been proven to have antimicrobial activity against micro organisms (Alinor, 2006). While some are powerful fish poison (Morah, 2004), others have synergistic effect (Jack and Nwachoka, 2015).

Not many plants have attracted wide applicability by man as *Bryophyllum pinnatum* L. of the family crassulaceae. It is commonly known as resurrection plant, Canterbury (cathedral) bells, air plant and miracle leaf. It is an erect, succulent perennial shrub about 1.5m high that can reproduce from seed and also vegetatively from leaf bulbils. The stem is thick and fleshy. The leaves are opposite fleshy, dark-purple and simple or trifoliate measuring about 10cm long and 5-6cm broad. *Bryophyllum pinnatum* has become naturalised in tropical and sub tropical areas and has been found to contain bufadienolide cardiac glycosides which can cause cardiac poisoning particularly in grazing animals (Mckenzie and

Dunster, 1986., Mckenzie et al. 1987). The plant has been recorded as being used as a traditional treatment for hypertension in Trinidad and Tobago (Lans, 2006) and also showed some insecticidal properties (Supratman et al., 2000). In Nigeria, it is employed by the Ijaws in several areas including the speeding up of the dropping of the umbilical cord of newly born babies (Okwu, 2007). The liquid from the leaf is also an excellent solvent for cleansing unripe plantain latex on the palm. It is the utility of this plant that aroused interest to investigate some of the constituents present in *Bryophyllum pinnatum* L. Soxhlet extraction of the fresh leaves with ethanol gave a crude viscous oil which on column chromatography eluted with 40-60°pet ether and  $\text{CH}_2\text{Cl}_2$  1:1 gave two products a pungent oil compound (1)  $R_f$  0.75 identified as 4-ethoxy - cyclopent-2-enone (1) and another oil (3)  $R_f$  0.52 also identified as 2,5-dimethoxyfuran by their spectra data such as infra red, mass and proton magnetic resonance spectra. The structure of the latter compound (3) has been confirmed by synthesis using furan (2) with bromine in methanol (scheme 1) and comparing the spectra of the synthetic compound with the isolated one. Both spectra showed (3) to be a mixture of isomers.

## II. MATERIALS AND METHODS

Infrared spectra were recorded on Pye Unicam SP 1050 spectrophotometer. Mass spectra were obtained on an AET, MS9 double focusing instrument at 2500C and 70 eV. Proton magnetic resonance spectra were measured in  $\text{CDCl}_3$  solution using TMS as an internal standard on a Varian HA 100 instrument with values given in  $\delta$ . TLC was performed on a silica gel GF254 plates using dichloromethane and petether 40 – 60° 1:1. All reagents were of Analar grade and solvents were purified by distillation before use.

### A. Extraction of *Bryophyllum Pinnatum*

300g of fresh *Bryophyllum Pinnatum* leaves obtained from Eagle Island in Port Harcourt were thoroughly cleaned by washing in running tap water and cut into shreds. The leaves were then put into a large soxhlet extractor in a 2 litre round bottom flask. After refluxing with 800ml absolute ethanol for six hrs, the flask and its content was allowed to cool to room temperature. The ethanol extract was distilled off using the rotary evaporator to give 7.5g of light

yellow viscous oil which gave two spots on tlc with  $R_{fs}$  0.75 and 0.52. The crude product was placed on a column of silica gel and eluted with  $CH_2Cl_2$  and pet ether 40 – 60<sup>0</sup> 1:1 to give 3.2g of a pungent oil compound (1) in 1.07% yield. Continuous elution with the same solvent also gave 2.5g of a light oil (compound 3) in 0.83% yield.

### B. Synthesis of compound (3)

In a 250ml three neck flask equipped with a stirrer, pressure equalizing funnel and a calcium chloride guard tube were placed 48ml dry methanol, 48ml dry benzene, 32g of anhydrous  $Na_2CO_3$  and 12.5ml fresh furan (2). The slurry was cooled to 0<sup>0</sup>C in an ice bath and an ice cold solution of 9ml of bromine in 84ml of methanol was added dropwise at a rate to maintain the temperature of 0<sup>0</sup>C. The mixture was stirred for additional two hours at 0<sup>0</sup>C after which it was filtered. The solid was washed with two 8ml portions of benzene. The filtrate was stirred with 8g of anhydrous  $MgSO_4$  for 30 min and filtered again. The bulk of the solvent was removed using the rotary evaporator and the white solid which separated was filtered off and washed with benzene. The filtrate was stirred with  $K_2CO_3$  for 20 min, filtered and distilled at reduced pressure to give an oil.

Yield 17.3g (70%)

I.r (film) 3100 – 3020, 2850 – 2810 $cm^{-1}$

P.m.r ( $CDCl_3$ ) H-2/5, 6.00s; 5.91s; H-3/4, 5.54s;  $OCH_3$ , 3.32s, 3.28s.

m.s  $M^+$  m/e 130; m/e 99; m/e 68

### III. RESULTS AND DISCUSSIONS

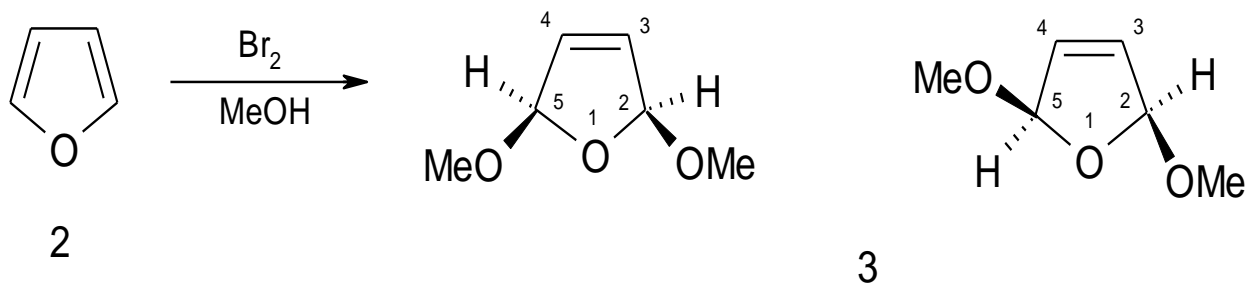
The infra red spectrum of the pungent oil (1) gave bands at 3100 – 3020. $cm^{-1}$  characteristic of the C-H stretching frequency of an  $Sp^2$  hybridized carbon. In addition, there were bands at 1745 $cm^{-1}$  and 1662 $cm^{-1}$  which are indicative of a five membered ring ketone and unsubstituted olefinic groups respectively. The presence of a ketonic group is further inferred when compound (1) formed yellow precipitate with 2,4-dinitrophenylhydrazine but failed to change fehling's solution to brick red. The mass

spectrum gave a molecular ion  $M^+$  at m/e 126 (50%) which was accurately mass measured to 126.1550 and corresponded to the molecular formula of  $C_7H_{10}O_2$ . The molecular ion fragmented by loss of carbon monoxide and ethoxide ion to give the fragment ions at m/e 98 and 82 respectively the latter being the base peak (Scheme 2). Other fragment ions are given in table 1.

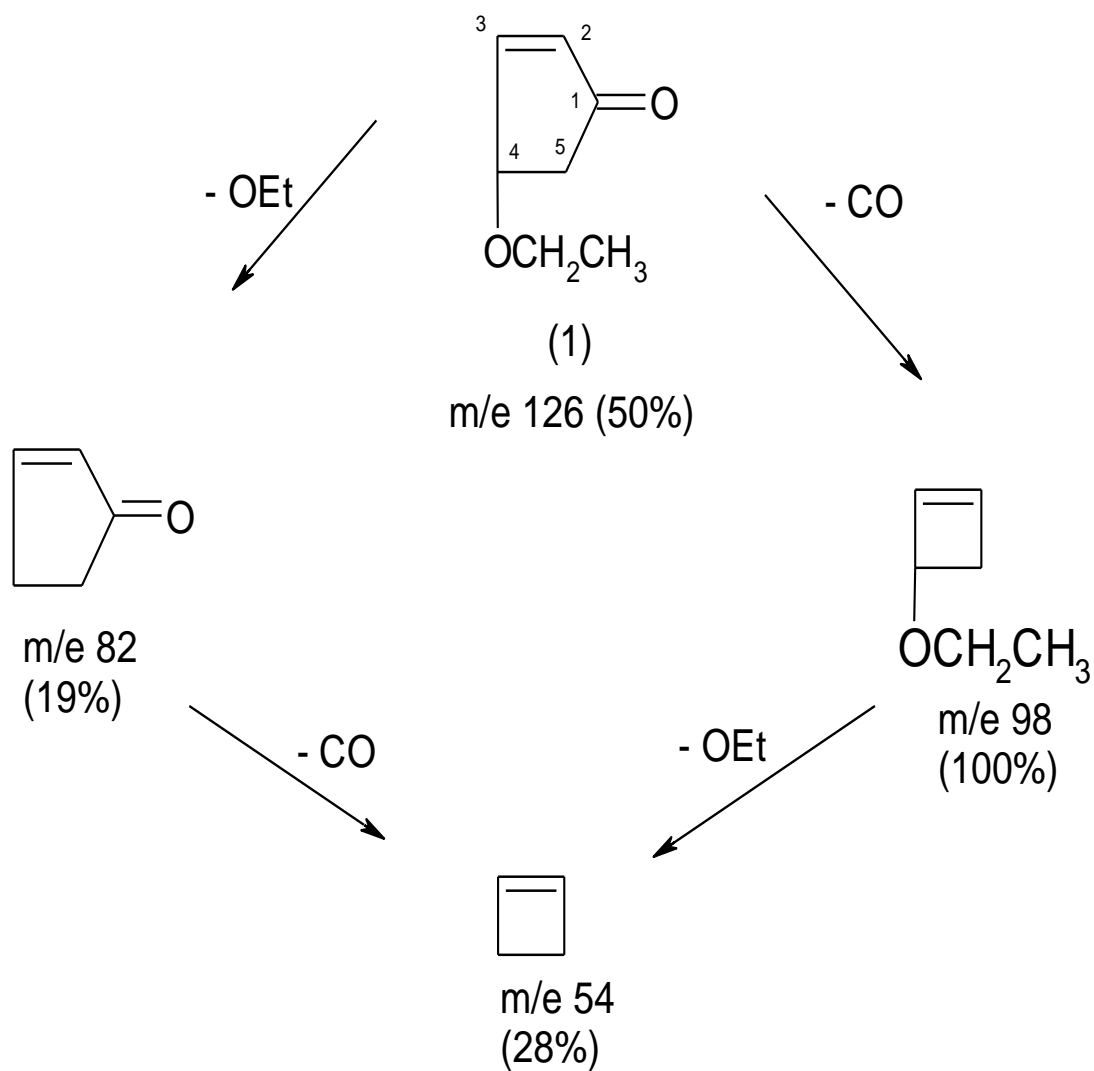
In the proton magnetic resonance spectrum, two olefinic protons H-3 and H-2 appeared at  $\delta$  7.52 and 6.16 respectively as double doublets with coupling constants  $J = 16$  and  $3H_z$ . A methine CH proton H-4 appeared at  $\delta$ 4.62 as multiplet being coupled to one of the olefinic and an adjacent methylene protons. There are two distinct methylene protons one appeared at  $\delta$ 2.59 as double doublet with  $J = 18$  and 8Hz and the other at  $\delta$ 2.20 double doublet with  $J = 18$  and 3Hz. The methylene protons of the ethoxy group appeared at  $\delta$ 3.51 as quartet with  $J = 8$  while the methyl protons next to the  $CH_2$  appeared at  $\delta$ 1.20 as triplet. This data corroborated evidence for the structure (1) suggested for this compound.

A very simple infra red spectrum of compound (3), gave a vinyl CH stretching at 3020 and 2850-2810 $cm^{-1}$  for the  $OCH_3$  group. In the mass spectrum of compound (3), a prominent molecular ion showed at m/e 130 (30%) which was also accurately mass measured to give 130.1160 and corresponded to the molecular formula of  $C_6H_{10}O_3$ . Two fragment ions at m/e 99 (45%) and m/e 68 (100%) were a result of sequential losses of two  $OCH_3$  groups (table 3).

The proton magnetic resonance spectrum of compound (3) showed clearly that it is a mixture of two isomers. H-2 and H-5 appeared at  $\delta$ 6.00 and 5.91 as singlets suggesting that they are in different chemical environments which precluded them from coupling to the olefinic protons at H-3 and H-4; so were the methoxy protons which occurred at  $\delta$ 3.32 and 3.25 also as singlets. The resonances of the H-3/4 protons coincided at  $\delta$ 5.45 as singlet.



Scheme 1



Scheme 2

**Table 1: Mass Spectrum of Compound (1)**

m/e	Ion	Abundance %
127	M <sup>+</sup> + 1	5
126	M <sup>+</sup>	50
98	M <sup>+</sup> - CO	100
82	M <sup>+</sup> - OEt	19
81		65
54	M <sup>+</sup> CO; -OEt	28

**Table 2: Mass Spectrum of Compound (3)**

m/e	Ion	Abundance %
131	M <sup>+</sup> + 1	3
130	M <sup>+</sup>	30
99	M <sup>+</sup> -OCH <sub>3</sub>	45
68	M <sup>+</sup> - 2(OCH <sub>3</sub> )	100

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