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Isolation of Novel 6-methylideneoxecane-3,4,5,7,8,9-hexol from the Leaves of *Rauwolfia vomitoria*, Apocynaceae

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Abstract

Rauwolfia vomitoria (Wennberg) belongs to the family Apocynaceae, the dried leaves were extracted successively using n-hexane, dichloromethane, 70% methanol, and concentrated *in vacuo*. Extracts were subjected to antibacterial assay and the butanol fraction was subjected to chromatographic purification to obtain NBF_{12} which was subjected to spectral analysis. The antibacterial of n-Hexane, dichloromethane, and 70% methanol extracts was inactive against the screened organisms assessed (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*). NBF_{12} is yellowish crystals (10.5 mg), R_f (0.69), UV, max Abs (248) The carbon ^{13}C-NMR spectrum displayed ten carbon atoms; one methylene, two methylene oxide carbon, 6-carbinolic carbon (Sp^3) and a quaternary carbon (SP^2). The spectrum showed a tertiary carbon at δ131.09 ppm, due to C-6 and at δ 114.74 ppm due to exocyclic methylidene carbon (Sp^2) ($C=CH_2$) linked to the C-6 position. Based on the spectral data NBF_{12} is 6-methylideneoxecane-3,4,5,7,8,9-hexol with a molecular formula $C_{10}H_{18}O_7$, molecular weight 250 g/mol.

Keywords: Isolation; Rauwolfia vomitoria; antibacterial; 6-methylideneoxecane-3,4,5,7,8,9-hexol.

INTRODUCTION

Rauwolfia vomitoria (Wennberg) belongs to the family Apocynaceae and is a medicinal plant used for the management of unknown pyrexia by the Ijaws of Southern Nigeria. This is due to its versatile utility in traditional medicine. It has been reported as an aphrodisiac, antimicrobial, antipsychosis, antihypertensive, antianxiety, and antioxidant effect (Etim et al., 2018; Emencheta et al., 2020; Oyeniran et al., 2020; Balogun and Akintunde, 2022). This medicinal plant has a battery of chemical constituents isolated and reported in the literature which includes; ursolic acid, sistosterol, stigmasterol, reserpine, reserpinine, deserpidine, ajmalicine, and ajmaline, 2,6-dimethoxybenzoquinone (Ajayi et al., 2021). The research aimed to determine the antibacterial effect and to characterize the chemical constituents of *R. vomitoria* leaves.

METHOD AND MATERIALS

Chemicals and Reagents

Methanol (Sigma U.K), Dichloromethane (Sigma U.K), n-hexane (Sigma U.K), Ethyl acetate (Sigma U.K), Dimethylsulphoxide (JHD), Sephadex LH-20 (Sigma), Silica gel 200-400 (Sigma U.K).

Equipment

UV spectrophotometry, NMR spectrophotometer 400 MHz (Agilent)

Microorganism

Staphylococus aureus NCTC6571, *Bacillus subtilis* NCTC8236, *Escherichia coli* ATCC25922, *Psuedomonas aeruginosa* ATCC 10145

Sample Collection

The leaves of *R vomitoria* were collected from the wild at Otabi Community in Oloibiri District, Ogbia Local Government Area, Bayelsa State, Nigeria.

Identification of Plant

The plant was identified and authenticated by Prof. A.T Oladele of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, and Herbarium specimen was deposited in the Herbarium of Pharmacognosy and Herbal Medicine and herbarium number (NDUP/24/01) was assigned to it.

Plant Preparation

The fresh leaves of the plant were washed and cut into small portions before air drying at room temperature for 14 days. The dried leaves were pulverized using the electrical blender to coarse powder, weighed, and stored in an airtight glass bottle.

Antibacterial Evaluation of the Extracts

The antibacterial evaluation of the extract was carried out according to (Balogun and Akintunde, 2022). *Staphylococus aureus* NCTC6571, *Bacillus subtilis* NCTC8236, *Escherichia coli* ATCC25922, *Psuedomonas aeruginosa* ATCC 10145. A loop full of bacteria strains was inoculated in Nutrient agar media and was incubated for 48 hours. 80 mL of sterile molten Muller Hinton Agar was transferred from the Mckonkey bottle to the Petri dishes and allowed to solidify. The bacteria strain was diluted equivalent to 0.5 Mcfarland standard in 5 mL of 0.9% normal saline and 0.03 mL of the active test strain was transferred to the solid media, swirled allowed to dry. Wells were bored on the agar using a sterile cork borer (9 mm), and the 100 mg/mL (0.1, 0.2, 0.3, and 0.4 mL) stock concentration of each of the extracts were prepared in dimethylsulphoxide and water in a ratio (3:7). 10, 20, 30, and 40 mg of each extract was used against the bacteria strain using Ciprofloxacin 5µg/disc as the standard drug and was incubated at 37ºC for 24 hours (Emencheta et al., 2020; Balogun and Akintunde, 2022; Karim et al., 2023; Dhital et al., 2024).

Isolation 6-methylideneoxecane-3,4,5,7,8,9-hexol

The methanol extract weighing 25 g was suspended in 200 mL of distilled water and it was partitioned sequentially using n-hexane, ethyl acetate, and n-butanol and the extracts were concentrated *in vacuo* using a rotary evaporator. The n-butanol fraction weighed 2 g was subjected to gel filtration using Sephadex LH-20 in a column (1.5 cm x 86 cm) and eluted with methanol (100%), 10 ml of the eluate collected to a total of 21 fractions coded (NBF 1-21). Based on the TLC profile NBF_{9-12} weighed 0.146 g was subjected to column chromatography using Silica gel as stationary phase (30 g, 200-400) with a dimension (1.5cm x 86 cm) and gradiently eluted with ethyl acetate, (100%); 95:5; 90:10; 80:20; 70:30; 65:35; 60:40; 50;50; 30:70; 10:90 (Ethyl acetate: Methanol) to 100% methanol and the progress of elution monitored using TLC in a solvent system ethyl acetate: methanol: water (100:16.5:13.5), fraction 12 gave a single spot on TLC, and concentrated *in vacuo* to give yellowish crystals weighed 10.5 mg which was coded NBF_{12}, subjected to spectroscopic analysis.

RESULT AND DISCUSSION

Antibacterial

Antibacterial evaluation of the n-hexane, dichloromethane, and 70% methanol extracts were screened at 10, 20, 30, and 40 mg and showed that the extracts were devoid of antibacterial activity because there was no zone of inhibition compared to the standard drug ciprofloxacin 5µg/disc which showed zone of inhibition; 24 mm, 21 mm, 22 mm and 21.5 mm against *S aureus*, *B subtilis*, *E coli* and *P aeruginosa* respectively, as illustrated in Table (1, 2 and 3). The results are in line with the report by Balogun and Akintunde, 2022 (Emencheta et al., 2020; Balogun and Akintunde, 2022).

Table 1. Antibacterial activity of n-hexane extract.

S/N	Agent/conc.	Zone of Inhibition (mm)			
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	10 mg	-	-	-	-
2	20 mg	-	-	-	-
3	30 mg	-	-	-	-
4	40 mg	-	-	-	-
5	Ciprofloxacin 5µg	24	21	22	21.5

Keys - = No zones of inhibition

Table 2. Antibacterial activity of dichloromethane extract.

S/N	Agent/conc.	Zone of Inhibition (mm)			
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	10 mg	-	-	-	-
2	20 mg	-	-	-	-
3	30 mg	-	-	-	-
4	40 mg	-	-	-	-
5	Ciprofloxacin 5µg	24	21	22	21.5

Table 3. Antibacterial activity of 70 % methanol extract.

		Zone of Inhibition (mm)			
S/N	**Agent/conc.**	***S. aureus***	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	10 mg	-	-	-	-
2	20 mg	-	-	-	-
3	30 mg	-	-	-	-
4	40 mg	-	-	-	-
5	Ciprofloxacin 5µg	24	21	22	21.5

Chemistry of 6-methylideneoxecane-3,4,5,7,8,9-hexol (NMR Analysis)

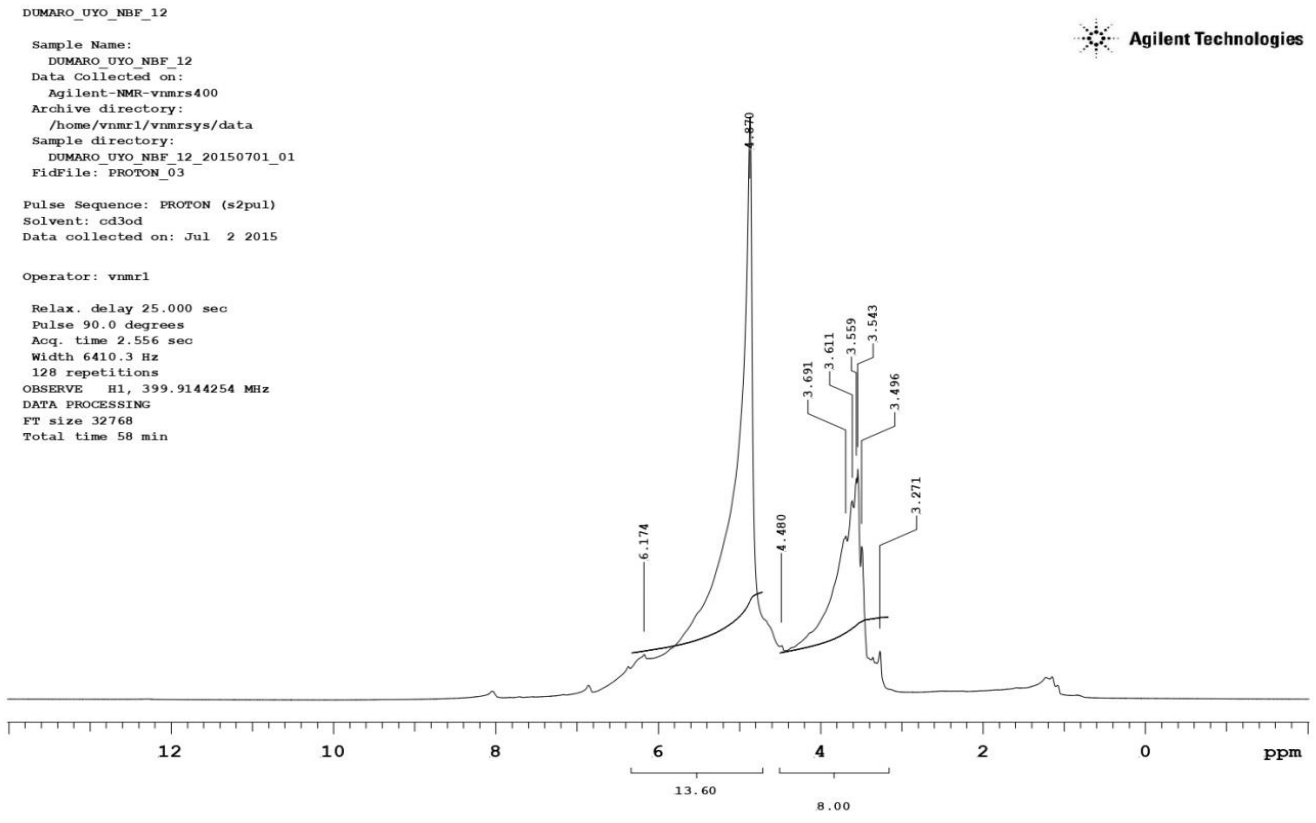

Figure 1. ^{1}H-NMR of Sample NBF_{12}

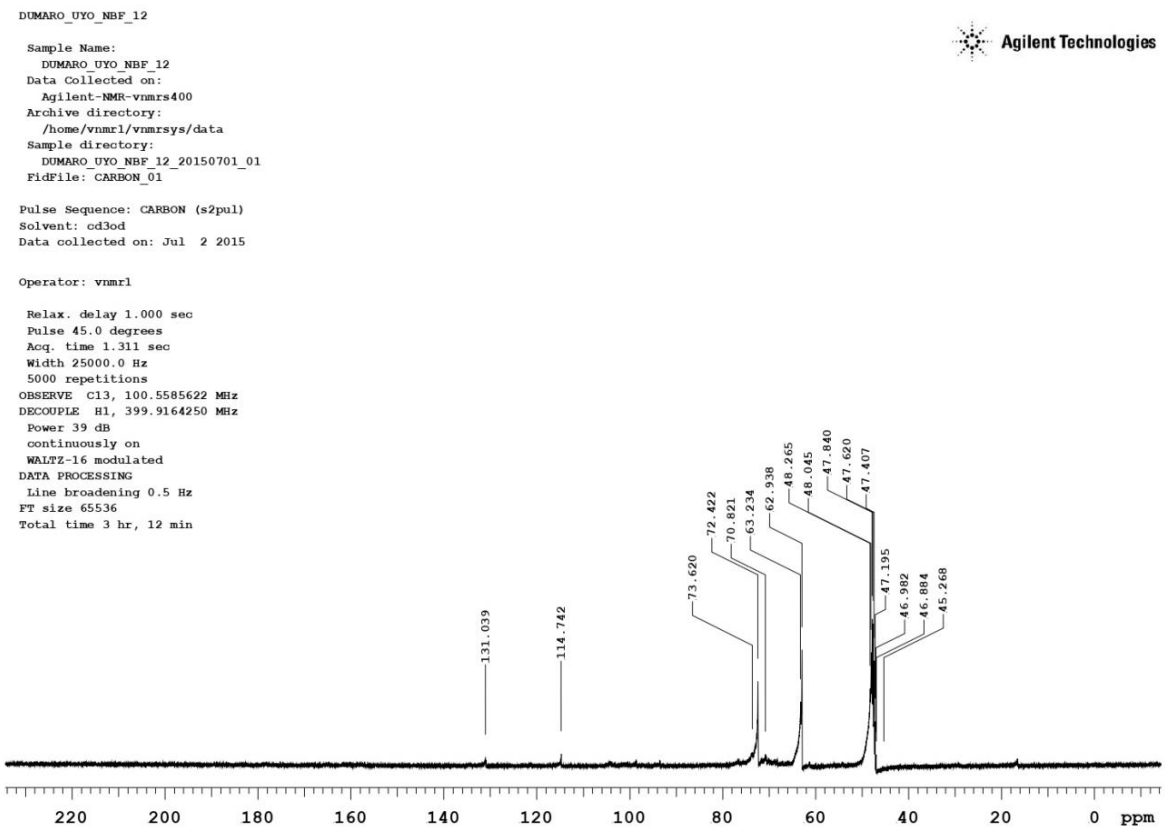

Figure 2. ^{13}C-NMR of Sample NBF_{12}

Yellowish crystals (10.5 mg), R_f (0.69), UV, max Abs (248),^{1}H-NMR (CD_3OD, 400 MHZ, δ, ppm); 6.1, (s, 2H), 4.87 (s, 6H, OH proton), 4.48 (s, 4H), 3.69-3.27 (m, 6H).

^{13}C-NMR (CD_3OD, 400 MHZ, δ ppm); 130.04 (C-6); 114.74 (=CH_2), 73.62 (C-4, C-8), 72.41 (C-2; C-10), 70.82 (C-5; C-7), 63.23 and 62.94 (C-3; C-9) respectively.

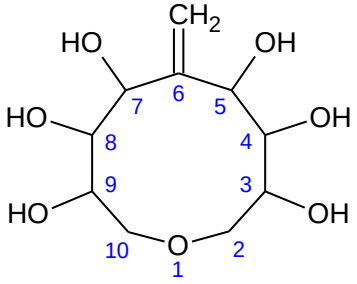

Figure 3. Chemical structure of 6-methylideneoxecane-3,4,5,7,8,9-hexol.

The proton NMR spectrum of sample NBF_{12} exhibited four prominent peaks. A downfield signal resonates at δ 6.17 ppm integrated for 2H and assigned to methylidene protons linked to C-6 due to the exocyclic, unsaturated (C=CH_2) group. An intense peak at δ 4.87 due to carbinol hydroxyl protons at (C-3; C-5and C-7; C-9) integrated for 6H protons and intense multiplet peak at δ 3.69-3.27 ppm due to methine (CH) of carbinolic proton at C-3-5; 7-9) positions integrated for six protons (6H) as shown in Figure 1.

The carbon ^{13}C-NMR spectrum displayed ten carbon atoms; one methylene, two methylene oxide carbon, 6-carbinolic carbon (Sp^3), and a quaternary carbon (SP^2). The spectrum showed a tertiary carbon at δ131.09 ppm, due to C-6 and at δ 114.74 ppm due to exocyclic methylidene carbon (Sp^2) (C=CH_2) linked to C-6 position, the signal at δ 73.62 due to C-4 & C-8 position. The signal at δ 72.42 ppm, due to (C-2 and C-10) methylene-oxide group, and are chemically equivalent. The carbinolic signal at δ70.82 ppm is due to C-5; C-7 and the signal at δ 63.23 & 62.94 ppm is due to C-3 and C-9 respectively as illustrated in Figure 2. Based on the foregoing, sample NBF_{12} proposed as 6-methylideneoxecane-3,4,5,7,8,9-hexol with a molecular formula $C_{10}H_{18}O_7$, molecular weight 250 g/mol (Kamentani et al., 1995; Bubb, 2003; Kalsi 2004; Sharma et al., 2005; Azogu, 2010). This is the first time of reporting this compound in *R. vomitoria* (Figure 3).

CONCLUSION

The extract was not active against the microorganism assessed and isolation from the n-butanol gave a novel 6-

methylideneoxecane-3,4,5,7,8,9-hexaol and the first time of reporting this compound from *Rauwolfia vomitoria*.

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***Author's Contribution*:** ADCO and ROR designed the work, ADCO and AF collected the Sample, ADCO, ROR, and AF carried out the isolation while DDE carried out the antibacterial assessment of the extracts. SJB performed a critical review and manuscript proofreading. The manuscript was written by ADCO and all authors read and approved the final copy.

***Competing Interest*:** The Authors have no competing interests.

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