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MACROLIDE FROM TETRAPLUERA TETRAPTERA ROOT

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ABSTRACT

The chloroform extract of Tetraplueratetraptera root gave a compound identified as Nnenoside A. The compound was isolated at chloroform: methanol ratio of 80:20 mixtures and the purity monitored with TLC and a single spot was observed using Pet. ether: chloroform: methanol ratio of 70:22.5:7.5% with Rf value of 0.833. The structure of compound was elucidated with combination of IR, ^{1}H and ^{13}C NMR, COSY, DEPT NMR and Mass spectral analysis.

Keywords: Isolation, characterisation, spectral analysis

INTRODUCTION

Plants contain untapped reservoir of bioactive compounds that can be used directly as well as 'lead' compound for synthetic ones. Many compounds of plant origin of which their active principle agents have been developed and currently in use include: Calabar bean (*Physostigmavenenisum)*, used traditionally as an ordeal poison from where methyl carbamate insecticides was developed; and pyrethrum insecticides from the flower of *Chrysanthemum cinerariaefolium* extract, which was discovered because of its local use to control insect pest (Oldfield, 1984). The root of Lonchocarpus is a source of rotenone used as poison to stun fish (Plotkin, 1988). Many drugs have also been developed of which their lead compounds were of plant origin e.g. penicillin. This therefore points out that the whole parts of the plant: fruits, flowers, leaves, stems, barks and roots can be potential source of drugs, pesticides and other compounds. These parts of plant contain secondary metabolites known as phytochemicals of which extracts can be toxic compounds that have potentials for use in development of natural pest control products (Agte*et al*, 1999; Dev and Karl, 1997; Saxena and Kidiavai, 1997 and Okogun 1983).

The composition and distribution of these plant constituents or active principles vary considerably from plant to plant. These include the saponins, flavonoids, alkaloids, tannins, phenols glycosides etc (Agunwa, 1996 and Mitscher, 1975). Phytochemicals are non-nutritive plant chemicals that have protective and disease preventive properties (Okwu, 2005 and Irvine, 1961). These chemicals act as deterrents to insects – pesticidal effects; they also have antioxidant property, hormonal action, stimulation of enzymes, antibacterial, antifungal and antimolluscidal effects etc (Close and Mcarthur, 2002; Aget*et al*, 2002).

Tetrapleuratetraptera (Fabaceae Family) is a medium sized deciduous tree with fern-like foliage and highly characteristic fruits. It is a perennial tree which grows along the Western Coast of Africa. The plant's common names are: Aiden (English), Ọshọhọ (Igbo), Aridan (Yoruba), Ighimiaka (Bini) and Edeminnangi (Efik). The tree reaches up to 4 m high and 1.5 - 3 m in girth. The leaves are sessile, glabrous or minutely hairy with common stalk 15 – 30cm long, slightly channelled on the upper surface. The pinnate are in mostly 5 – 9 pinnae, 5 – 10cm long, mostly opposite but sometimes alternate, 6 – 12 leaflet on each side of the pinna-stalk always alternate, 12 – 25 mm long, 6 – 12 mm broad, slightly elongated elliptic or slightly obovate, rounded at both ends, the apex sometimes slightly notched, the base usually unequal, practically glabrous, with slender stalks about 2 mm long.

The flowers are pinkish-cream turning to orange and are densely crowded in spike racemes 5 – 12 cm long, usually in pairs in the upper leaf axes. The fruit is persistently hanging at the end of branches on stout stalks, 15 – 25 mm long by about 5 cm across the wing-like ribs; dark purple-brown, glabrousand glossy, usually slightly curved. Two of the wings are hard and woody and the other two filled with a

soft sugary pulp. The seeds are hard, black, flat, oval, about 8 mm long, embedded in the body of the pod which does not split. The wood is reddish to brown heart wood, fairly hard, sapwood white (Keay and Onochie (1964).

In Nigeria, it is used for numerous purposes (Adewunmi*et* al., 1993). The powdered fruit is used as fish poison and in ointment for the treatment of skin diseases (Bode *et al.*, 1996 and Adesina, 1982). The intensive odour produced when the fruit is roasted is claimed to repel insects and snakes (Nwaiwu and Aka, 1986). The methanol extract of the fruit which was linked to their saponin content has been reported to have molluscidal property and its mechanism of action is by ultrastructural effects of the snail digestive system (Bode *et al.*, 1996 and Adesina*et al.*, 1980). The plant is used in West Africa to flavour soups and taken as general tonics and stimulant or as part of postpartum diet therapy (Okwu, 2005). The leaves, bark, roots, fruits and kernels are used for medicinal purposes (Okochi, *et al.*, 1999; Nwaiwu and Aka, 1986 and Adesina, 1982).

The reputed efficacies of these plants have been experienced and passed on from one generation to the other. In fact, lack of enough scientific proof of efficacies claimed by local people in Nigeria called for this study. It is the ultimate aim of this research to isolate and characterise the bioactive compound in this locally used plants.

MATERIALS AND METHODS

Sample Collection and Preparation

*Tetrapleuratetraptera*plant root was gotten from UmuntuOlokoroUmuahia South Local Government Area of Abia State, Nigeria. This was identified by Mr IbeNdukwe of the Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State.

The root of *Tetrapleuratetraptera*plant was reduced with sharp knife into smaller parts to facilitate drying. These samples were air-dried at room temperature. The air-dried samples were pulverized into powdery form using a brand new corona landers manual milling machine which was stored in labelled polyethene bags.

Extraction of Sample

The extraction method used in this study was a modification of JohnBull (2001a and b, JohnBull and Abdu, 2006). 750g to 1kg of each of the pulverized samples were soaked in glass bottles to obtain the ethanol (crude) extract by percolating in about 2.5 litres of ethanol (98% analytical standard) for 72hours, followed by filteration and then concentrated under reduced pressure rotary evaporator at 34^{o}C separately (JohnBull*et al*, 2001a and b). These were then allowed to stand for complete evaporation of the remaining ethanol at room temperature. The crude ethanol extracts was stored in labelled covered beakers for further analyses.

Partitioning of the Crude Ethanol Extract

Part of each of the crude ethanol extracts of the samples was subjected to partitioning.15 - 20 g of the crude ethanol extract of the sample was partitioned using 150ml/150ml v/v of chloroform and water. The sample was first dissolved using the solvents. This was continuously stirred to ensure complete dissolution and was then transferred into a separating funnel which was shaken until homogenous mixture was obtained. It was then allowed to stand for 24hrs before separating. The chloroform extracts was allowed to evaporate completely under room temperature.

Column Chromatography of Chloroform fraction

3.0g of chloroform fractions was mixed with 30g of silica gel (50 – 200 mesh) to get homogenous solid mixture slurry and loaded on a silica gel packed column. 3g of silica gel was then added to protect the sample applied. The column was eluted with different solvent mixture gotten from the manipulation of the ratio mixture, starting with 100% (100ml) petroleum ether. Then varying the mixture ratio at 5ml interval with chloroform i.e. (95ml Pet.ether and 5ml chloroform, followed by 90ml Pet.ether and 10ml chloroform) etc, until it got through to 100% (100ml) chloroform. Thereafter, methanol was mixed in like manner with chloroform until there was complete elution. The eluateswere collected in fraction of 100ml. Each fraction was evaporated to dryness and transferred

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into a labelled 100ml beaker and covered with foil. The fractions were monitored on TLC and the one that gave single spots were selected and set aside for spectroscopic analyses.

IR was recorded on Perkin Elmer FT Model. Readings were taken between 4000 cm^{-1} and 625 cm^{-1}. MASSSpectrometry (MS) was performed on a Finnigan MAT Incos-XL mass spectrometer operating under electron impact (EI) ionization mode at 70 eV. ^{1}H NMR spectra were recorded on a Bruker AM-500 FT-NMR spectrometer operating at 500 MHz, while ^{13}C NMR (broad band and DEPT; proton decoupled at 125 MHz on a Bruker AM-500 spectrometer. Spectra were recorded as 12-20 mmol/L solutions in $CDCl_3$ at ambient temperature. Chemical shifts were expressed in parts per million (ppm) (δ) relative to external tetramethylsilane. Coupling constants *J* are in Hz. The 13C NMR spectral assignment was made partly through DEPT, HMQC (heteronuclear multiple quantum coherence) and HMBC (heteronuclear multiple bond connectivity) and partly through a comparison of the chemical shifts with the published data for similar compounds. Assignments of protons were based on COSY-45, decoupling and NOESY experiments. The purity of the compounds was monitored on TLC with silica gel PF 254. In these NMR experiments, after the pulse there is a short delay, during which the decoupler is turned off, and the ^{13}C NMR spectrum becomes modulated by the CH coupling frequency. After the delay the decoupler is turned on, and the FID is recorded. If the delay is 1/J then the quaternary and CH_2 carbons are positive, and the CH and CH_3 signals are negative. If the delay is 1/2J all peaks except quaternary are nulled.

DEPT(Distortionless Enhancement of Polarization Transfer): The DEPT technique has proven superior to others in providing information on attached protons reliably, efficiently and with high selectivity. It is a proton-carbon polarization transfer method, so DEPT spectra are actually more sensitive than normal acquisitions. A set of spectra with pulse delays adjusted for B/2 (DEPT-90) and 3B/4 (DEPT-135) are taken. The DEPT-90 spectrum shows only CH carbons, the DEPT-135 shows positive CH_3 and CH, and negative CH_2 signals. It is important to understand that the appearance of positive and negative signals can be reversed by phasing, so it is necessary to have some way of determining whether the spectrum has been phased for CH_2 positive or negative. Quaternary carbons are invisible. "Leakage" can occur in DEPT-90 spectra because 1*J*C-H varies as a function of environment, and the technique assumes that all 1*J*C-H are identical. This can result in small peaks for CH_2 and CH_3 signals, which should have zero intensity. For similar reasons the C-H of terminal acetylenes (C/C-H) will show anomalous intensities in DEPT spectra (either nulled or very small in DEPT-90, or present in DEPT-135) because the CH coupling is much larger (around 250 Hz) than the normal value of 125 Hz for which the DEPT experiment is usually parameterized. To further prove this structure and to assure that these assignments are correct, data were compared with ^{13}C-NMR data in literature for compounds with similar structures.

RESULTS AND DISCUSSION

This compound was isolated at chloroform: methanol ratio of 80:20 mixtures. The purity of the compound was monitored with TLC and a single spot was observed using Pet. Ether: chloroform: methanol ratio of 70:22.5:7.5%. A single spot was observed in TLC having Rf value of 0.833. This was then subjected to spectral analysis.

The vibrational frequency of compound isolated from the root of *Tetrapleuratetraptera*plant showed characteristic absorption at 2988.83cm^{-1} which is very prominent for aliphatic C-H stretching vibration. Nevertheless, there appeared a weak absorption at 3430cm^{-1} which is a very evident peak for the vibration frequency of an enclosed secondary amine. Carbonyl (C=O) frequency absorption in this compound A was prominent at 1718.79 cm^{-1}. The C-H deformation appeared at the beginning of the finger print region with values of 1441 cm^{-1} and 1358.88cm^{-1}. Other functional groups in the finger print region were observed as listed in Table 1.

The structure was further established using NMR employing 2D NMR including ^{1}H – ^{1}H COSY and DEPT spectra. The chemical shifts of the NMR spectral analyses are shown in Table 2, for ^{1}H NMR and Table 3 for ^{13}C NMR. The ^{1}H NMR spectrum showed chemical shift at ∂ 2.2 and 2.4 (singlet, down field) which are methyl groups of deshielded oxygen on C-28, C-29 and sugar moiety. Unshielded methyl group always resonate at ∂ 1.1 to 1.5. The methyl group at C-21 showed

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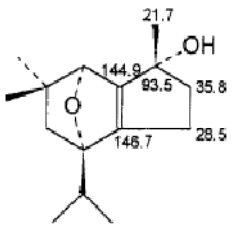
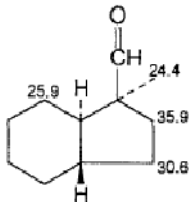
prominently as a singlet at ∂ 1.4. The other chemical shifts are characteristics of steroidal skeleton and sugar as well as pyrolidinyl moiety. The pattern of the chemical shift of the sugar skeleton is prominently observed between ∂ 3.2 – 4.8 always as doublet of doublet and doublet. The peaks between ∂ 0.6 – 0.9 are protons of methylene groups in the cyclic system with a significant amount of strain (five membered ring). The ^{13}C NMR (Table 3) further elucidated the various assignments given to each carbon. Furthermore, the chemical shifts of C-12, C-15, and C-16 at 22, 29, and 34 ppm are also comparable with those of the corresponding carbons in **x** and **z** found in literature.



x



z

By the help of DEPT spectra, it was easy to distinguish between the quartenary, methyl, methylene and methine carbons. Whereas the methylene carbons are inverted, quartenary, methyl and methine carbons are in the normal phase of the X-axis of the spectrum. A combination of ^{13}C and DEPT 135 NMR revealed the presence of one methyl, thirteen methylene, twelve methine and nine methoxy, four methinoxy and seven quartenary carbons. The ^{1}H – ^{1}H Correlation spectra which showed despartial arrangement as well as position of the various protons associated with compound A. This pattern is not divergent from already established ^{1}H – ^{1}H COSY of similar compounds with steroidal substituted sugars (disaccharides).

This was substantiated by the mass spectral analysis gave a molecular ion peak of m/z 893 suggesting a molecular formula $C_{54}H_{64}O_{19}N_2$ with a base peak of m/z 275. The fragmentation pattern showed a loss of a sugar moiety m/z 225; gave a fragment ion at m/z 668.6 from the molecular ion. A further cleavage with subsequent loss of m/z 180 resulted in a fragment ion peak of m/z 488. Other possible fragmentation pattern is shown in figure 1.

On the basis of above corroboration coupled with the fact that the spectra data were compared with those in literature, therefore, the structure Nnenoside A was proposed.

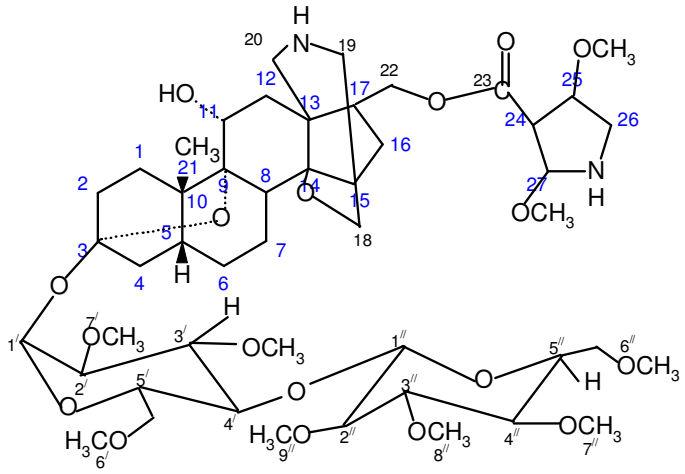


Figure 1.Structure of Nnenoside A

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Table 1: Infrared Absorption Bands for the Compound from the root of Tetrapleuratetraptera

Absorptions Peaks (cm^{-1})	*Group*	*Remarks*
3430	N – H stretching	Secondary Amine
2988.83	C – H stretching,	Aliphatic groups
2918.87	$-CH_2 - CH_2-$, CH_3	
2880	(Bending vibration deformation)	
1718.79	C=O	Carbonyls
1441.81		
1358.88	CH_3 and CH_2	Alkanes
1159.37		
1111.14	C – O – C. C -O – H	Ethers, alcohols, sugar
1053.27	C- N, C – O	Aliphatic Amines
852.85	N – H	
810		

Table 2. ^{1}H NMR Chemical Shift of the Compound from the root of Tetrapleuratetraptera

∂-Shift	*No of protons*	*Multiplicity*	*Assignment*
0.6 – 0.9	2H	d	H-26
1.0	2H	t	H-1/2/4/6/7/15/16
1.4	3H	s	H-21
1.6	1H	t	H-17
2.2	3H	q	H-28/29
2.4	2H	s	H-22
2.8	2H	t	H-18
3.3	3H	t	H-$1'/2'/3'$
3.4	3H	s	H-$1''/2''/3''$
3.7	3H	s	O –H
4.0 - 4.5	Poorly resolved		Sugar, $-CH_2$, $O-CH_3$
5.4	1H	d	O-H, N-H

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Table 3.^{13}C NMR Chemical Shift of the Compound from the root of Tetrapleuratetraptera

Carbon No./Assignment	*∂-Shift*	*Multiplicity*	*Carbon No./Assignment*	*∂-Shift*	*Multiplicity*
1	22	CH_2	24	38	CH
2	32	CH_2	25	53	CH
3	77	CH	26	59	CH_2
4	43	CH_2	27	55	CH
5	35	C	28	38	OCH_3
6	18	CH_2	29	38	OCH_3
7	31	CH_2	$1^{/}$	77	CH
8	31	C	$2^{/}$		CH
9	55	C	$3^{/}$		CH
10	37	C	$4^{/}$		CH
11	77	CH	$5^{/}$		CH
12	22	CH_2	$6^{/}$	78	OCH_3
13	39	C	$7^{/}$		OCH_3
14	77	C	$8^{/}$		OCH_3
15	29	CH_2	$1^{//}$	77	CHO
16	34	CH_2	$2^{//}$		CH
17	41	CH	$3^{//}$		CH
18	14	CH	$4^{//}$		CH
19	59	CH_2	$5^{//}$		CH
20	59	CH_2	$6^{//}$	78	OCH_3
21	19	CH_3	$7^{//}$		OCH_3
22	39	CH_2	$8^{//}$		OCH_3
23	78	C	$9^{/}$		OCH_3

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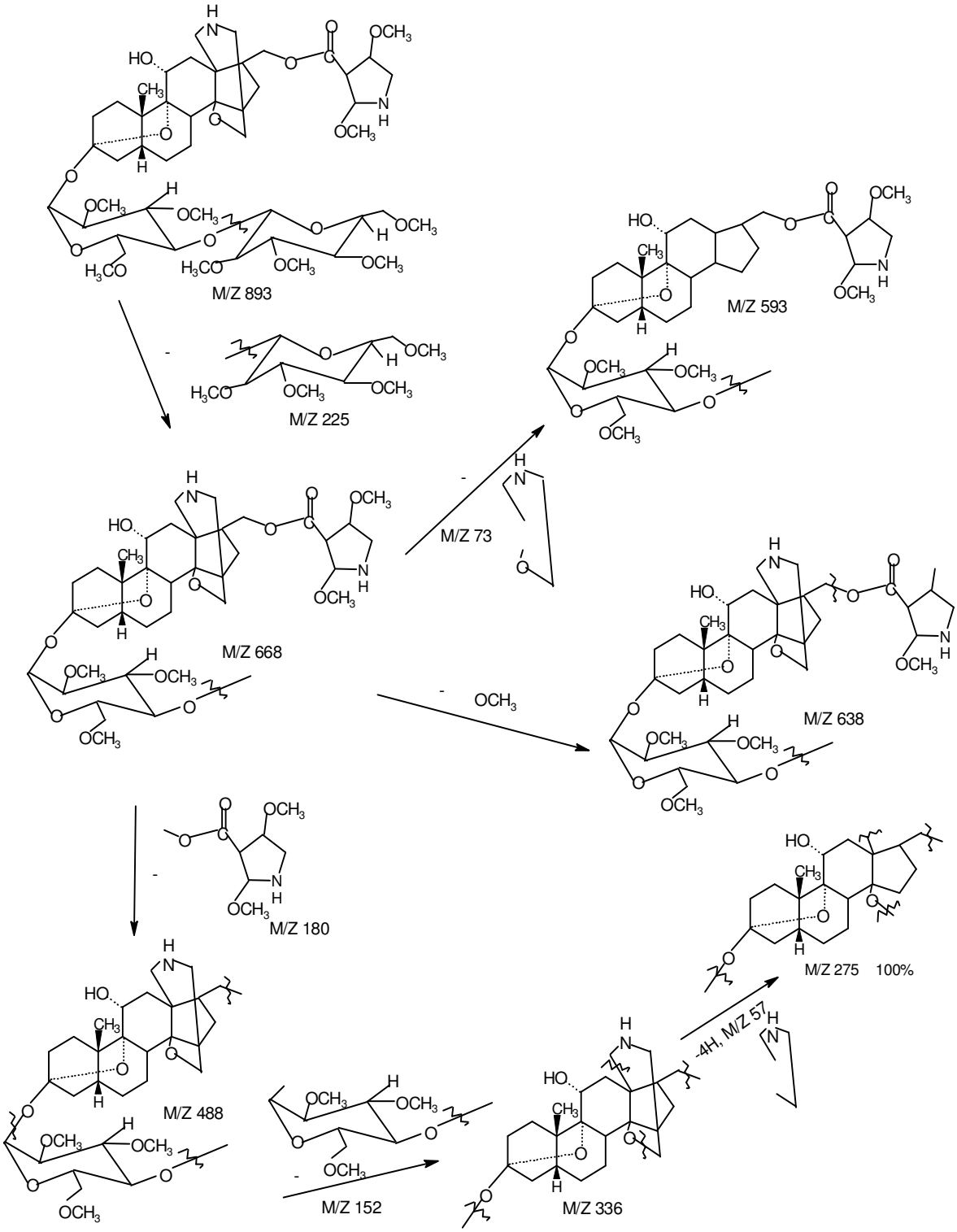

Figure 1.Fragmentation Pattern of Nnennoside A

CONCLUSION

The bioactive compound isolated from the part the plant under study contains a steroidal nucleus. Many steroidal based compounds of plant origin have also been isolated. This bioactive compound could be explored for its potentials in disease management, pesticidal activities and related uses.

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