Structural Characterization of ZS – 2A: An Antiplasmodial Compound Isolated from *Zizyphus spina-christi* Root Bark

Bulus Adzu[1,2,*], Abdu Kaita Haruna[2], Mohammad Ilyas[2], Umar Usman Pateh[2], Florence David Tarfa[3], Ben Ahmed Chindo[1] and Karniyus Shingu Gamaniel[1]

[1]Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria; [2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria and [3]Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria

Abstract: *Zizyphus spina-christi* (Rhamnaceae) is a popular medicinal plant that grows wildly in Asia and Tropical Africa. The plant is widely used in ethnomedical practice for the treatment of fever. As a step towards the isolation of biologically active constituents of this plant, we carried out a bioassay guided extraction of the root bark using solvents of varying polarity including, hexane, chloroform, ethylacetate and methanol. An antiplasmodial compound, designated as ZS-2A, was isolated from the chloroform extract and the chemical structure of the compound was characterized using UV-visible, IR, ^{13}C and ^{1}H NMR and thermo-analytical techniques. Our analysis established ZS – 2A as a betulinic acid.

Key Words: ZS-2A, spectroscopy, thermo-analysis, betulinic acid.

INTRODUCTION

Medicinal plants have been the major sources of drugs and lead compounds for drug synthesis. *Zizyphus spina-christi* (Rhamnaceae) is widely used in ethnomedical practices for the management of a plethora of diseases including fever [1, 2]. Studies on the efficacy of the root bark of this important medicinal plant is in progress in our laboratories [3-6]. ZS-2A is a bioactive compound isolated from the chloroform extract and reported to have antiplasmodial properties against *Plasmodium berghei berghei* infected mice [5]. To our knowledge, the chemical structure of ZS-2A has not been reported in literature. The present study was therefore designed to elucidate the structure of ZS-2A using spectrometric techniques (UV-visible, IR, ^{13}C and ^{1}H NMR) as well as thermo-analysis.

MATERIALS AND METHODS

Plant Material

Zizyphus spina-christi Willd–Sp.Pl. ed 4 [Willdenow] 1 (2): 1105 1798 [Jul 1798] (IK) (*The International Plant Names Index www.ipni.org*) were collected from Midlu, Adamawa State, northeastern Nigeria. The plant was identified and assigned voucher specimen number 4108, and was deposited at the herbarium of the Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja, Nigeria.

*Address correspondence to this author at the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria; Tel: +234 8084848802; E-mail: bulusadzu@yahoo.com

Isolation of ZS-2A

The dried root bark was milled into powder and (1.29 kg) sequentially extracted with solvents of varying polarity using a soxhlet extractor (Quickfit, England), to give hexane (4.6 g), chloroform (27.8 g), ethylacetate (4.4 g) and methanol (245 g) extracts. The chloroform extract (10 g) was subjected to flush column chromatography (Silica gel, Aldrich Chemicals 230 – 400 Mesh) using hexane – ethylacetate as eluent and monitored with TLC on precoated plates (Whatman K5, 150A 250µ, USA). Spots on TLC were visualized under UV light (254/365 Eagle Scientific Ltd, UK). ZS – 2A was eluted in the process with mixtures of hexane and ethylacetate (50:50%) [5].

Spectrometric Analysis

UV- Visible

The UV-Visible spectra of ZS – 2A (1 mg) in 10 ml ethanol [7] were recorded on UV-160A, Shimadzu Co., Japan. Absorptivity was scanned from (λ_{max}) 200 – 600nm.

IR Spectrum

The IR spectra were taken as KBr pellets on Nicolet® IR100, Thermo Electron Corporation, UK.

NMR Spectroscopy

The ^{13}C and ^{1}H NMR spectra were recorded on Mackintosh HD: MacNMR 250 mHz and mercury BB 200 mHz spectrometer with residual $CDCl_3$ as internal reference. The sample was dissolved in CD_3OD. The chemical shifts were reported in δ (ppm) and coupling

constant in Hz. Further analysis were performed using the Distortionless Enhancement by Polarisation Transfer (DEPT) analysis for proton attachment, Heteronuclear Multiple Bond Correlation (HMBC) experiments for weak proton - carbon coupling (^{1}H - ^{13}C) and ^{1}H - ^{1}H Correlation Spectroscopy (COSY) coupling constants.

Thermo-Analysis

The melting point of ZS – 2A was established using the Differential Scanning Calorimeter (DSC) technique [8]. This was performed with a DSC instrument (NETZSCH DSC 204F1, USA); set to scan the sample from 0 – 500^{o}C.

RESULTS

The UV spectrum of ZS-2A shows absorption at 211nm. The IR spectrum showed prominent frequencies at 1457.78, 1696.45, 2853.78 – 2925.42 and 3583.66 cm^{-1} (Figure **1**). The ^{13}C NMR spectrum is presented in Figure (**2**). The DEPT spectrum of the compound revealed occurrences of six CH_3 (δ 78.8, 55.5, 50.6, 49.3, 47.1 and 38.4 ppm); eleven CH_2 (δ 109.5, 38.9, 37.2, 34.4, 32.3, 30.6, 27.9, 27.0, 25.6, 21.0 and 18.4 ppm) and six CH (δ 27.9, 19.3, 16.1, 15.9, 15.4 and 14.7 ppm) protons (Figure **3**). The ^{1}H NMR showed two methylene protons at δ 4.62 and 4.75, a secondary methyl signal at δ 1.70 ppm, and two oxygenated protons (Figure **4**). The ^{1}H – ^{1}H COSY shows that the olefinic protons coupled with each other, and the methyl multiplet at δ 1.70 (Figure **5**). The melting point of ZS-2A is 314^{o}C.

DISCUSSION

Spectroscopy techniques, which include the UV-visible, IR and NMR spectroscopy, have been a versatile analytical tool in chemistry. They provide infor-"mation on the qualitative and quantitative composition of compound molecular specie especially when used with other analytical tools. ZS – 2A showed a UV-visible absorption at 211nm. Carbonyl chromophore in various functional groups, characteristics of the n → n*, n → δ* and n→n * transitions are known to absorb around that wavelength. The IR spectra showed absorption characteristics to $–CH_3$ (asymmetry), C==O (stretching α, β –unsaturated), $–CH_2$ (asymmetric stretching), $–CH_2$ (in plane scissor motion) and –OH (stretch) [9, 10]. The IR spectrum suggests that among the functional moieties in ZS-2A are carbonyl and hydroxyl groups.

Peak finding results for: Bulus 2a 09/11/2007
Frequency: 400.00 - 3999.64, threshold: 10.231, sensitivity: 50.00
Peak finding result table:

Peak#	1	2
Position	2925.42	1457.78
Height	0.117	4.332

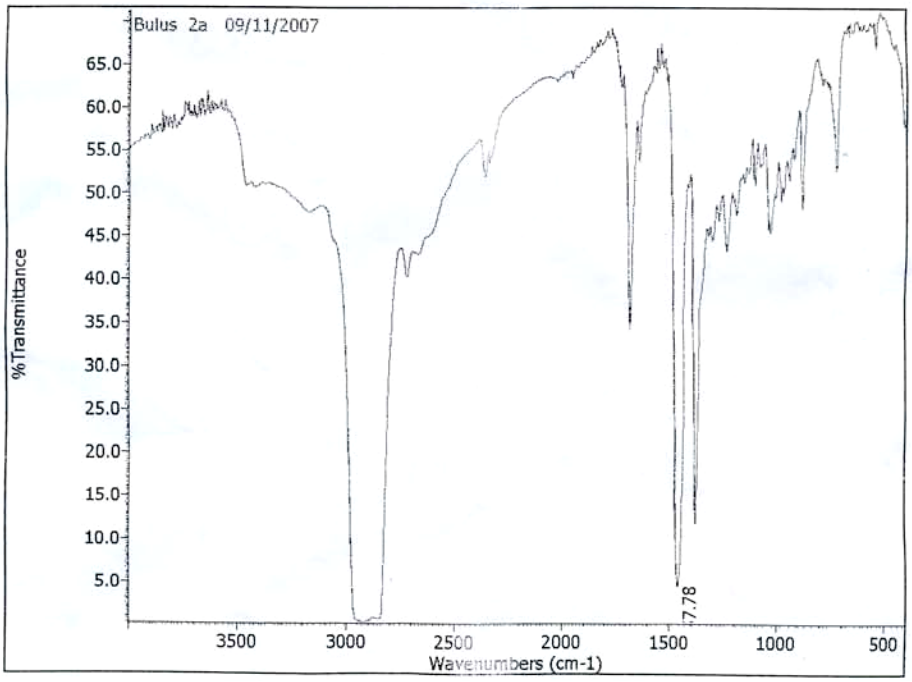


Figure 1: IR spectrum of ZS-2A.

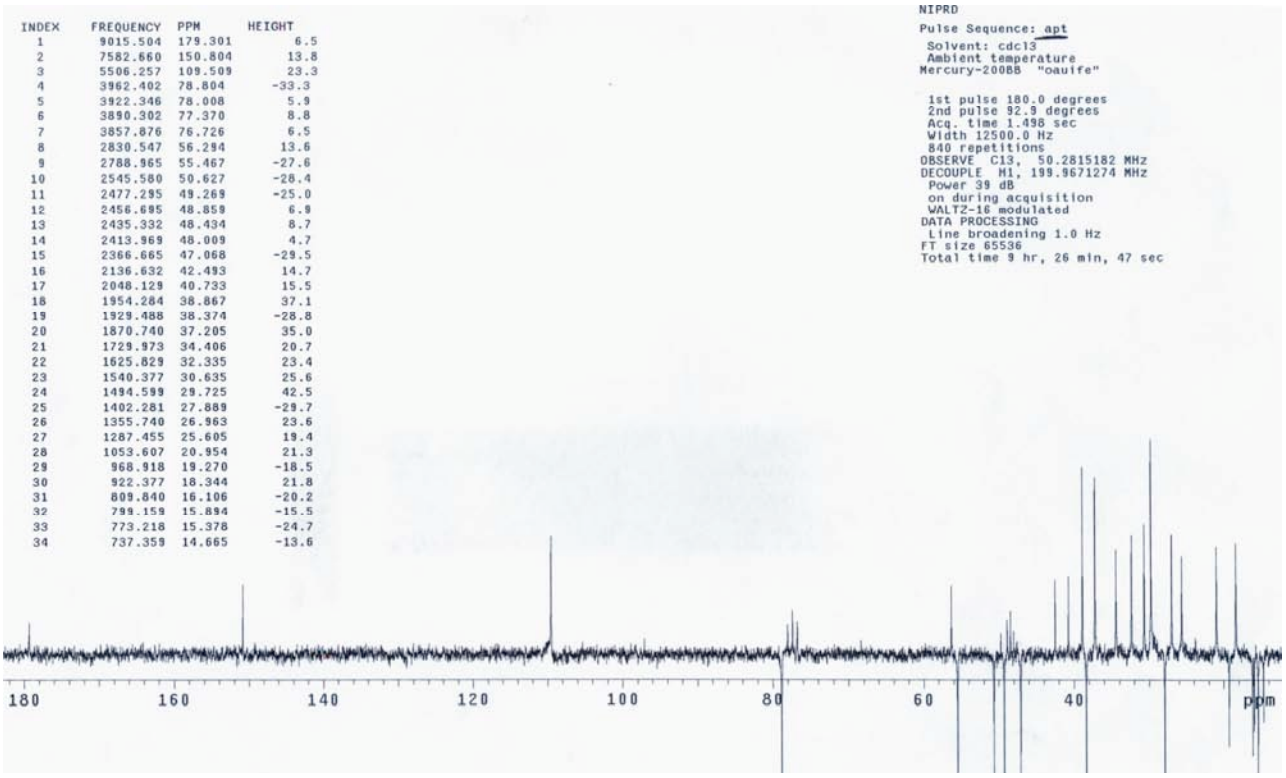


Figure 2: ^{13}C NMR spectrum of ZS-2A.

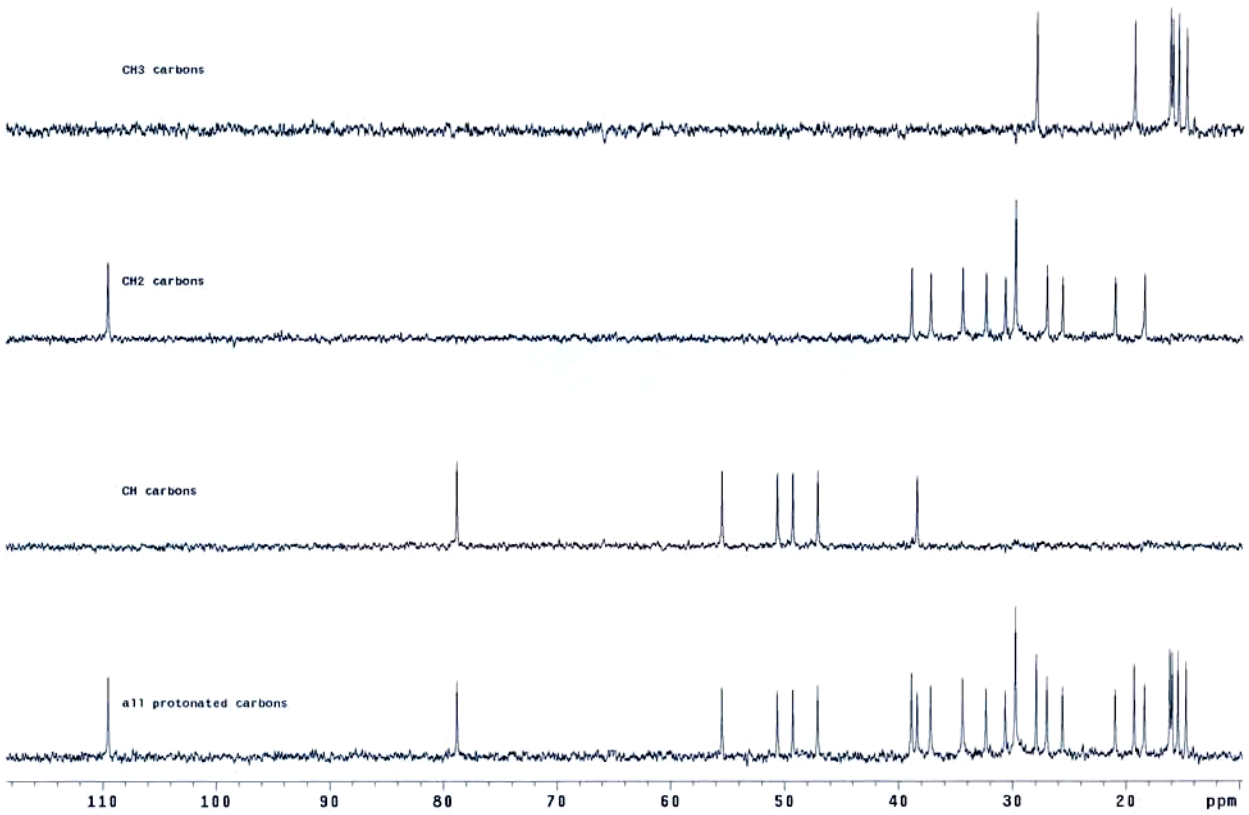


Figure 3: ^{13}C NMR DEPT analysis of ZS-2A.

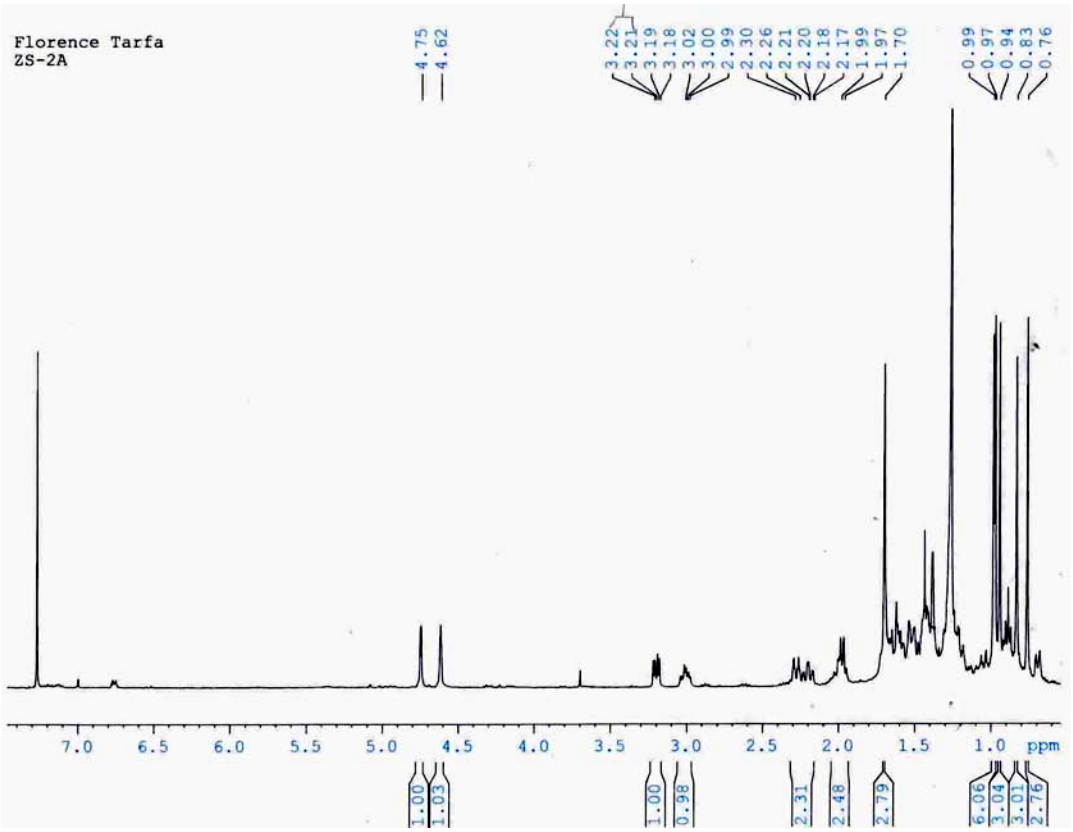


Figure 4: ^{1}H NMR spectra of ZS-2A.

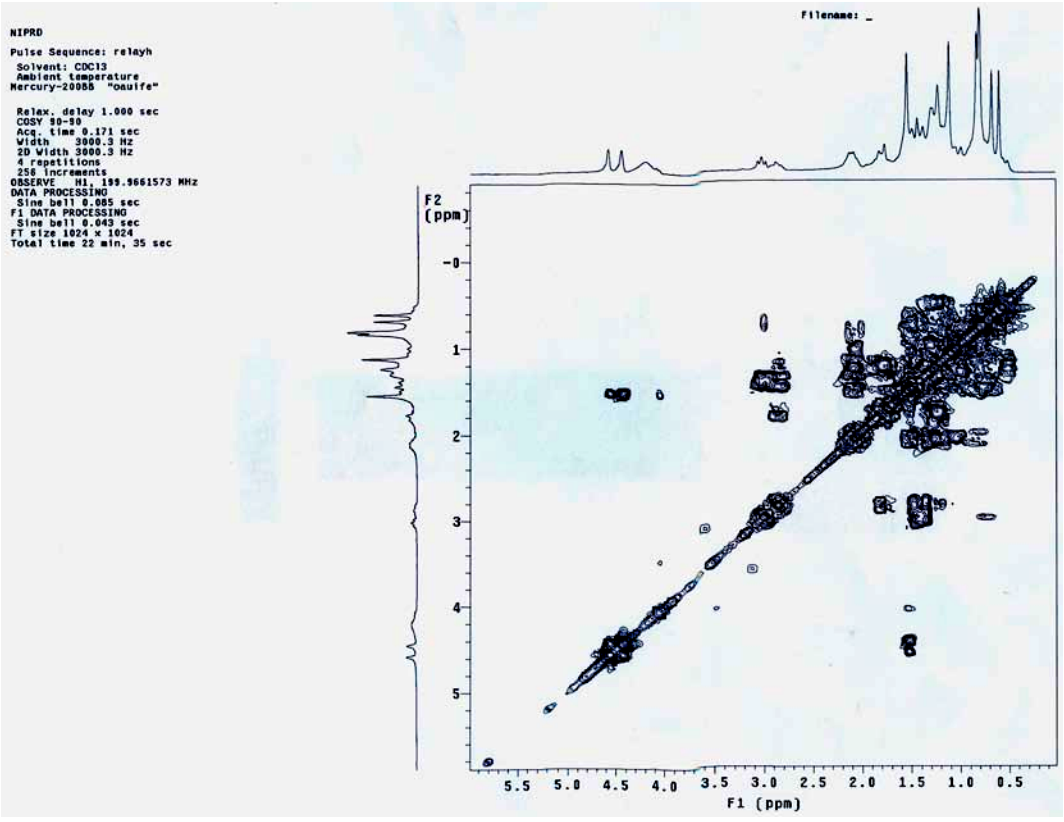
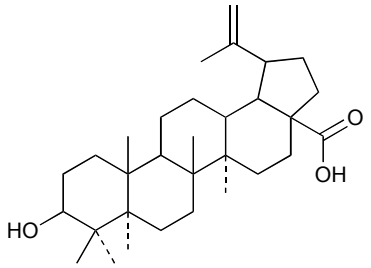


Figure 5: ^{1}H – ^{1}H COSY of ZS-2A.

The ^{13}C NMR spectra of ZS-2A revealed resonances due to 34 C-atoms including 2 attributable to the solvents used ($CDCl_3$ and CD_3OD, with δ 48 and 78 ppm respectively). The DEPT spectrum revealed the presence of 23 protonated carbons clearly observed as 6 $-CH_3$ groups, 11 $-CH_2$, 6 –CH. The ^{13}C NMR showed absorption at δ 179.3 ppm characteristic of carbonyl signal (Shaari and Waterman, 1996). The ^{13}C NMR as well as the DEPT spectrum properties analysis could be accommodated with comparisons with literature data given for pentacyclic triterpenes isolated from plant materials [11-15]. The two double bond protons, along with the secondary methyl signal in the ^1H NMR is attributable to the isopropylene residue of the skeletal moiety. No aromatic protons were observed (δ 6 – 8) as confirmed by the IR spectra.



Betulinic acid

ZS – 2A is thus deduced as betulinic acid on the basis of the assignment of these ^{13}C and ^1H NMR signals aided by the DEPT, HMBC and COSY experiments; the UV and IR data; the melting point and comparisons with literature. Betulinic acid, also known as Mairin, is a naturally occurring pentacyclic triterpene widely distributed in the plant kingdom. It has been shown to exhibit variety of biological activities including being shown to have anti-retroviral, anti-inflammatory, anticancer and antimalarial properties [16-18]. Interestingly, ZS-2A exhibited antiplasmodial effect [5]. These justify the current interest in betulinic acid for its development as a potential antiplasmodial agent [19, 20].

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