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Full Length Research Paper

Synthetic modification of gedunin and comparative antibacterial activity of gedunin and 7-deacetoxy-7α-hydroxygedunin potassium salt

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Gedunin, a highly oxidized triterpenoid was obtained from hexane extractive of stem wood of *Entandrophragma angolense* (Welw) C. D. C. of the family Meliaceae. The gedunin was dissolved in minimum methanol at 40°C and set aside in the dark for recrystallization. Pure gedunin crystals were obtained in 0.92% yield. Methanolic alkaline hydrolysis of the gedunin afforded 7-deacetoxy-7α-hydroxygedunin potassium salt in 90.53% w/w yield. The gedunin and 7-deacetoxy-7α-hydroxygedunin potassium salt were screened against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* using broth microdilution assay method. 7-Deacetoxy-7α-hydroxygedunin potassium salt had minimum inhibitory concentration (MIC) of 1000 µg/ml against *K. pneumoniae,* 2000 µg/ml against *S. aureus*, and *E. coli*. However, *B. subtilis* was not susceptible to 7-deacetoxy-7α-hydroxygedunin potassium salt at 2000 µg/ml. Gedunin was not active against all the bacterial organisms tested at 2000 µg/ml.

Key words: Gedunin, synthesis, gedunin potassium salt, antibacterial, broth microdilution method.

INTRODUCTION

Natural products continue to be an important global source of therapeutically effective medicines; about 75% of anti-infective drugs are of natural origin (Newman et al., 2003). The anticancer drug Paclitaxel (1), an alkaloid from the Pacific Yew tree *Taxus brevifolia* and the antimalarial artemisinin (2) a terpenoid from *Artemisia annua* are examples of drugs derived from plants. The importance of synthetic modifications of drugs is exemplified in the conversion of artemisinin into the more stable and bioavailable artesunate (3) and artemether (4), and in other drugs derived from natural products (Haynes and Simone, 1994; Cragg et al., 1997; Newman et al., 2003), as shown in Figure 1.

Hence, the use of artemisinin combination therapies (ACTs) is for the treatment regimen of resistant malaria as recommended by the World Health Organization (WHO). Gedunin (5) is a bioactive natural product with potential for development into a drug. It is obtainable from renewable raw materials such as *Entandrophragma angolense* (Welw) C.D.C., *Cedrela sinensis* Juss, *Azadirachta indica* (Neem) and most plants of the family Meliaceae (Khalid et al., 1989; Bray et al., 1990). Gedunin is a highly oxidized triterpenoid (24, 25, 26, 27-tetranortriterpenoid) with multiplicity of functional groups. It was first isolated from the light petroleum ether extractive of the heartwood of *E. angolense* (Akisanya et al., 1960). Gedunin and some of its related compounds

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have been reported to possess a wide range of biological activities, such as antimalarial (Guo et al., 2005), antifeedant, insecticidal (Schwinger et al., 1983; Kraus et al., 1994; Alessandra et al., 2006), antifungal (Sundarasivarao and Madhusudhanarao, 1977), anti-prostate cancer (Haley et al., 2006; Shaikh et al., 2007), antileishmanial (Hay et al., 2007), anti-HIV and as inhibitor of colon cancer cells (Shaikh et al., 2007). Mahmoodin (6), a derivative of gedunin has been reported to possess antibacterial activity (Siddiqui et al., 1992).

The growing demand for active antimicrobial drugs due to increasing resistance to currently used antibiotics has necessitated search from renewable natural sources.

Amit and Shailendra (2006) recommended that the structure activity relationships of gedunin limonoids be pursued so that they can be safely introduced into our arsenal of pharmaceuticals to safeguard the humanity from the wrath of disease and its discomfort.

The aim of this work was to obtain gedunin from the stem wood of *E. angolense* (Welw) C.D.C. carry out repeat synthesis of 7-deacetoxy-7α-hydroxygedunin potassium salt (7) from the gedunin and investigate their antibacterial potential.

MATERIALS AND METHODS

Nuclear magnetic resonance spectroscopy (NMR)

^{1}H and ^{13}C NMR data were obtained using BrukerAvance III 500 and Avance 500 asc with ascend magnet both of 500 MHz at the Columbia University, USA. Gedunin was dissolved in deuterated chloroform ($CDCl_3$) and gedunin potassium salt was dissolved in deuterated water (D_20).

Melting point determination

Melting points of compounds were determined using Barnstead Electrothermal melting point apparatus (Model 9100).

Reagents and chemicals

Hexane (analytical grade), ethyl acetate (analytical grade), methanol (analytical grade), vanillin (analytical grade), were purchased from Sigma Aldrich (Germany).

Plant material

The heartwood of *E. angolense* used in this investigation was collected from Iyere in Owo Local Government area of Ondo State, Nigeria. The plant was identified at the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Extraction and isolation of gedunin

The dried pulverized stem wood of *E. angolense* (3 kg) was packed into an aspirator bottled and extracted by percolation, refluxing with n-hexane 6 L at 60°C for 48 h. The extract was vacuum filtered with Whatman No. 1 filter paper. The filtrate was concentrated under vacuum with rotary evaporator at 40°C to 100 ml and allowed to stand in the dark for 24 h during which gedunin crystallized out. The isolation of the gedunin was guided by thin layer chromatography (TLC) using authentic gedunin sample as reference. The gedunin crystals designated OK-S2 were collected by filtration dried in desiccator and recrystallized from methanol in 0.92% w/w yield which had melting point of 219 to 220°C. This melting point was undepressed by the addition of authentic gedunin sample which had melting point of 219 to 220°C.

Thin layer chromatography

TLC guided isolation of gedunin from hexane extractive of stem wood of *E. angolense* was performed on precoated normal phase TLC plate (K5, Whatman) previously activated at 115°C for 2 h. The mobile phase consisted of hexane-ethyl acetate (3:1). Authentic gedunin was dissolved in ethyl acetate and used as reference. After development, the plate was air-dried and sprayed with 1% w/v vanillin sulphuric acid reagent. The sprayed plates were heated at 105°C for 2 min. The chromatogram showed gedunin as a purple spot for both sample and reference at R_f value of 0.35.

Preparation of 7-deacetoxy-7α-hydroxygedunin potassium salt

Gedunin potassium salt was prepared as reported by Okogun and Orishadipe (1996). Briefly, gedunin 11 g (0.0225 M) was weighed accurately and dissolved in 130 ml of aqueous methanolic potassium hydroxide (5.5 g, 0.0975 M). It was then warmed with swirling in a water bath at 55°C until it dissolved. The swirling was continued in the water bath for a further 20 min without bringing the solution to boil. The solution was left to stand at room temperature, (crystallization sets in within an hour). After 4 h at room temperature, white crystals of 7-deacetoxy-7α-hydroxygedunin potassium salt (7) designated OK-S3 were collected by vacuum filtration using Whatman No. 1 filter paper and sintered glass funnel. The crystals were washed with ice cold methanol and dried in a desiccator. Yield was determined to be 9.9581 g (90.53%). ^{1}H and ^{13}C NMR spectra of 7-deacetoxy-7α-hydroxygedunin potassium salt were obtained.

Bacterial strain

Over night broth cultures of the typed test organisms namely *Staphylococcus aureus* (S2014 Sigma-Aldrich), *Escherichia coli* ATCC 25922 and clinical isolates of *Bacillus subtilis,* and *Klebsiella pneumoniae* were diluted to10^{7} cfu/ml monitored by spectrophotometric methods as described by Dominguez et al. (2001). Two to three colonies of 20 h growth on Mueller-Hinton Agar of the organisms to be studied were suspended on 50 ml pre-warmed (37°C) Mueller-Hinton broth. The suspension was incubated overnight at 37°C, diluted 1/2500 in the same pre-warmed medium and incubated in water bath with agitation (50 rpm). The absorbance of the culture was monitored with a spectrophotometer (6405 Jenway, Barloworld Scientific Ltd. Dunmow, Essex CMB 3LB), using 450 nm wavelength and 1 cm cuvette until absorbance of 0.1 was reached and a plotted standard curve (equivalent 2.5 - 3.0 × 10^{7}cfu/ml for *E. coli* and 1.8 - 2.0 × 10^{7} cfu/ml for *S. aureus* and *B. subtilis*). The experimental and controls were done in duplicates.

Antibacterial test

The minimum inhibitory concentrations (MICs) of the compounds were determined by broth microdilution method (BMM). 2 mg of each compound was dissolved in 0.2 ml of dimethyl sulfoxide (DMSO) and made up to 1 ml with 0.8 ml of the sterile

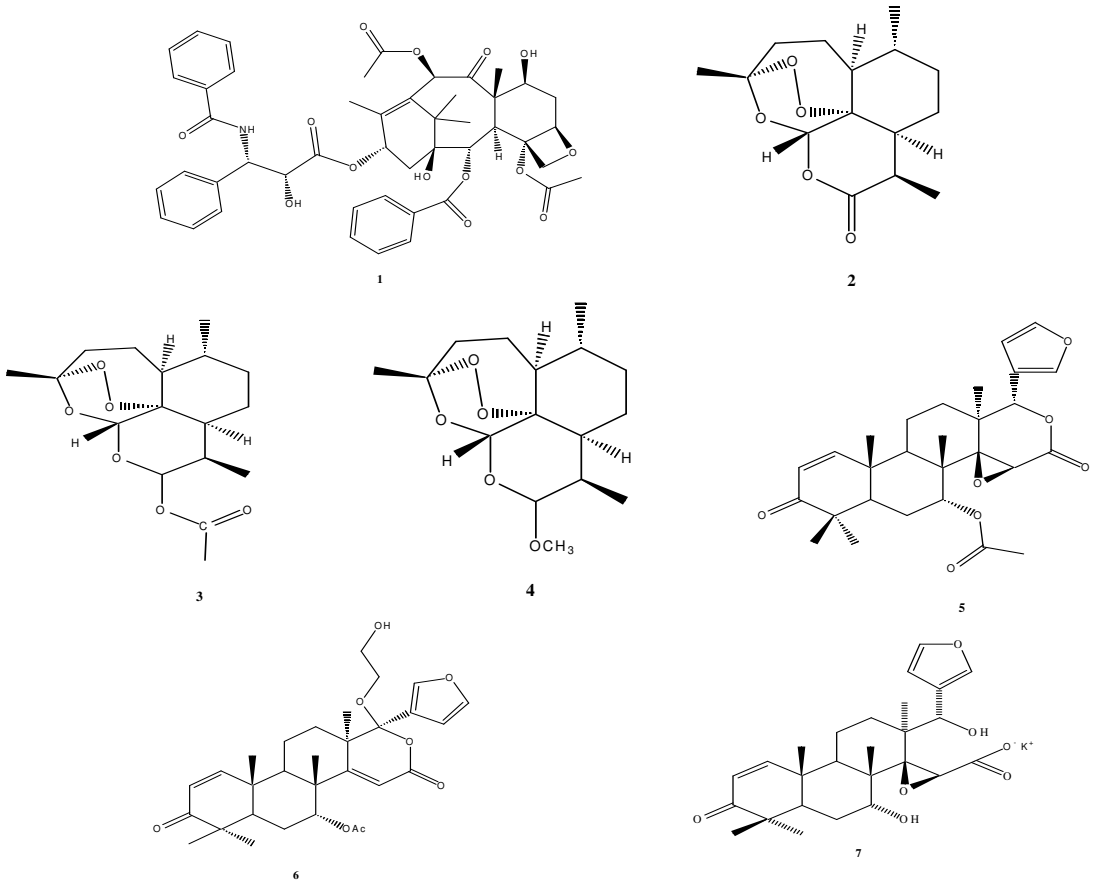

Figure 1. Structure of (1), Paclitaxel; (2), artemisinin; (3), artesunate; (4), artemether; (5), gedunin; (6), mahmoodin; (7), 7-deacetoxy-7α-hydroxygedunin potassium salt.

Mueller-Hinton nutrient broth to give stock concentration of 2000 µg/ml. Then, 100 µl of each test compound solution was transferred to the first designated well of a sterile 96 well microtiter plate in duplicate. 50 µl of the sterile nutrient broth was pipetted into Wells 2 to 12. Wells A1 - A12 and B1 - B12 were used for organism control. Wells G1 - G12 and H1 - H12 were used for standard drug control. Using a multi-channel pipette (Finnpipette II, Fisherbrand 50 - 300 µl), 50 µl of solution in Well 1 was transferred to Well 2, mixed thoroughly by pipetting up and down four times, and the process repeated through to Well 12 where 50 µl was withdrawn and discarded. All the wells were inoculated with 50 µl of overnight diluted cultures of each organisms (10^7 cfu/ml) prepared by spectroscopic method as described earlier. The plates were incubated for 24 h at 37°C. Standard drug used was Ciprotab (Fidson, Lagos Nigeria), at stock concentration of 500 µg/ml. MIC was determined as the last concentration where no growth was observed. The presence or absence of growth of organism was observed using macroscopic inverted mirror (Oladosu, 2011). All assays were carried out in duplicate.

RESULTS AND DISCUSSION

Extraction and isolation of gedunin

Gedunin (5) from the hexane extractive of *E. angolense* stem wood and recrystallized from methanol was obtained in yield of 0.92% w/w. It had melting point of 219 to 220°C. The melting point was undepressed by the addition of authentic gedunin sample. Its ^{1}H and ^{13}C chemical shift δ-values are consistent with that of gedunin previously reported (Gonzalez et al., 1989; Duddeck and Dietrich, 1992).

Alkaline hydrolysis of gedunin

Gedunin was converted to 7-deacetoxy-7α-hydroxygedunin potassium salt (7), by methanolic alkaline hydrolysis as previously reported (Okogun and Orishadipe, 1996). The product was obtained in 90.53% yield. It decomposed at 180°C with characteristic effervescence due to release of furfural.

^{1}H and ^{13}C NMR spectra analyses of gedunin

The ^{1}H and ^{13}C NMR spectra assignment for OK-S2 were in agreement with those reported for gedunin by Gonzalez et al. (1989) and Duddeck and Dietrich (1992). ^{1}H NMR spectrum of OK-S2 showed the presence of five

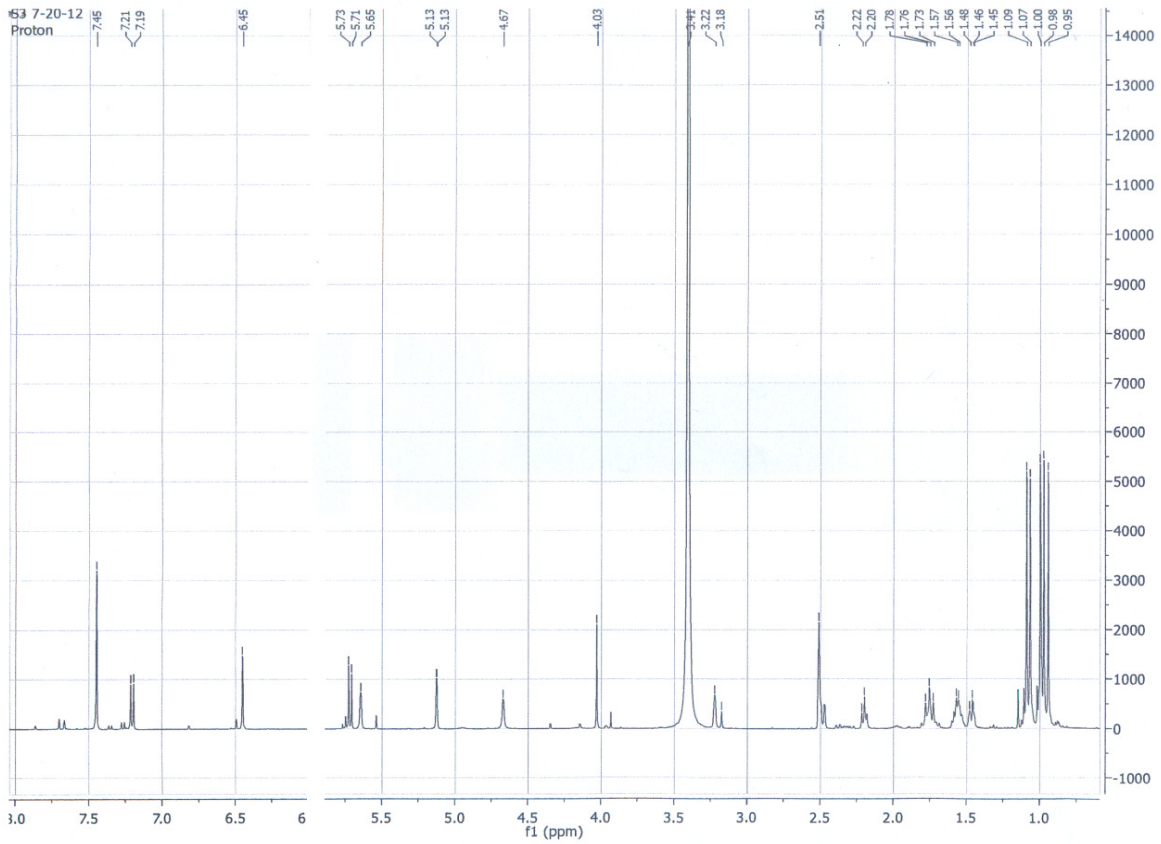

Figure 2. Full ^{1}H NMR Spectra of 7-deacetoxy-7α-hydroxygedunin potassium salt.

tertiary methyl protons δ 1.09 (3H, s, H-28), 1.10 (3H, s, H-29), 1.18 (3H, s, H-30), 1.25 (3H, s, H-19) and 1.28 (3H, s, H-18), one acetate methyl proton 2.13 (3H, s, H-32) each for three protons; three methylene protons 1.96 (1H, dd, H-6α), 1.84 (1H, dd, H-6β), 2.03 (1H, m, H-11α), 1.87 (1H, m, H-11β), 1.59 (1H, m, H-12α), 1.73 (1H, m, H-1β), ten methine protons 7.12 (1H, s, H-1), 5.89 (1H, s, H-2), 2.21 (1H, d, H-5), 4.55 (1H, s, H-7), 2.52 (1H, dd, H-9), 3.56 (1H, s, H-15), 5.64 (1H, s, H-17), 6.37 (1H, s, H-22) and at δ 7.44 for two accidentally isochronous protons (H-21 and H-23), with total number of signals accounting for 34 hydrogen atoms.

The ^{13}C NMR spectra of gedunin showed nine quaternary carbons at δ 204.09 (C=O, C-3), 44.06 (C, C-4), 42.65 (C, C-8), 40.04 (C, C-10), 38.75 (C, C-13), 69.75 (C-O, C-14), 167.42 (O-C=O, C-16), 120.44 (C, C-20),169.88 (O-C=O, C-31); ten methine carbons at δ156.91 (HC=C, C-1), 126.03 (HC=C, C-2), 46.06 (CH, C-5), 73.24 (HC-O, C-7), 39.56 (CH, C-9), 56.91 (HC-O, C-15), 78.27 (HC-O, C-17), 143.09 (HC=C, C-21), 109.87 (HC=C, C-22), 141.21 (HC=C, C-23); three methylene carbons at δ 23.28 (CH_2, C-6), 15.00 (CH_2, C-11), 26.03 (CH_2, C-12); five tertiary methyl carbons at δ17.75 (CH_3, C-18), 19.74 (CH_3, C-19), 27.18 (CH_3, C-28), 21.07 (CH_3, C-29), 18.32 (CH_3, C-30); and one acetate methyl carbon at δ21.91 (CH_3, C-32

The ^{13}C and ^{1}H chemical shift δ-values assignment for gedunin are identical to those reported by Gonzalez et al. (1989) and Duddeck and Dietrich (1992). The^{1}H NMR proton resonances at δ 7.44 (lH, s, H-21), δ 6.37 (lH, s, H-22) and δ 7.44 (lH, s, H-23) are characteristic of the β-substituted furan ring protons H-21, H-22 and H-23 of gedunin. From the heteronuclear single quantum coherence (HSQC) spectrum, the corresponding furan ring carbon resonances occurred in the ^{l3}C NMR spectrum at δ 143.09 (C-21), δ 109.87 (C-22) and δ 141.21 (C-23). C-20 was a quaternary carbon at δ 120.44. The ^{1}H NMR showed the characteristic epoxidic 15α-H at δ 3.56 for gedunin.

^{1}H and ^{13}C NMR spectra of 7-deacetoxy-7α-hydroxygedunin potassium salt

The ^{1}H and ^{13}C NMR spectral data of 7-deacetoxy-7α-hydroxygedunin potassium salt were consistent with that of gedunin potassium salt (7) as reported (Okogun and Orishadipe, 1996). The ^{1}H and ^{13}C NMR spectra of 7-deacetoxy-7α-hydroxygedunin potassium salt are shown in Figures 2, 3 and 4.

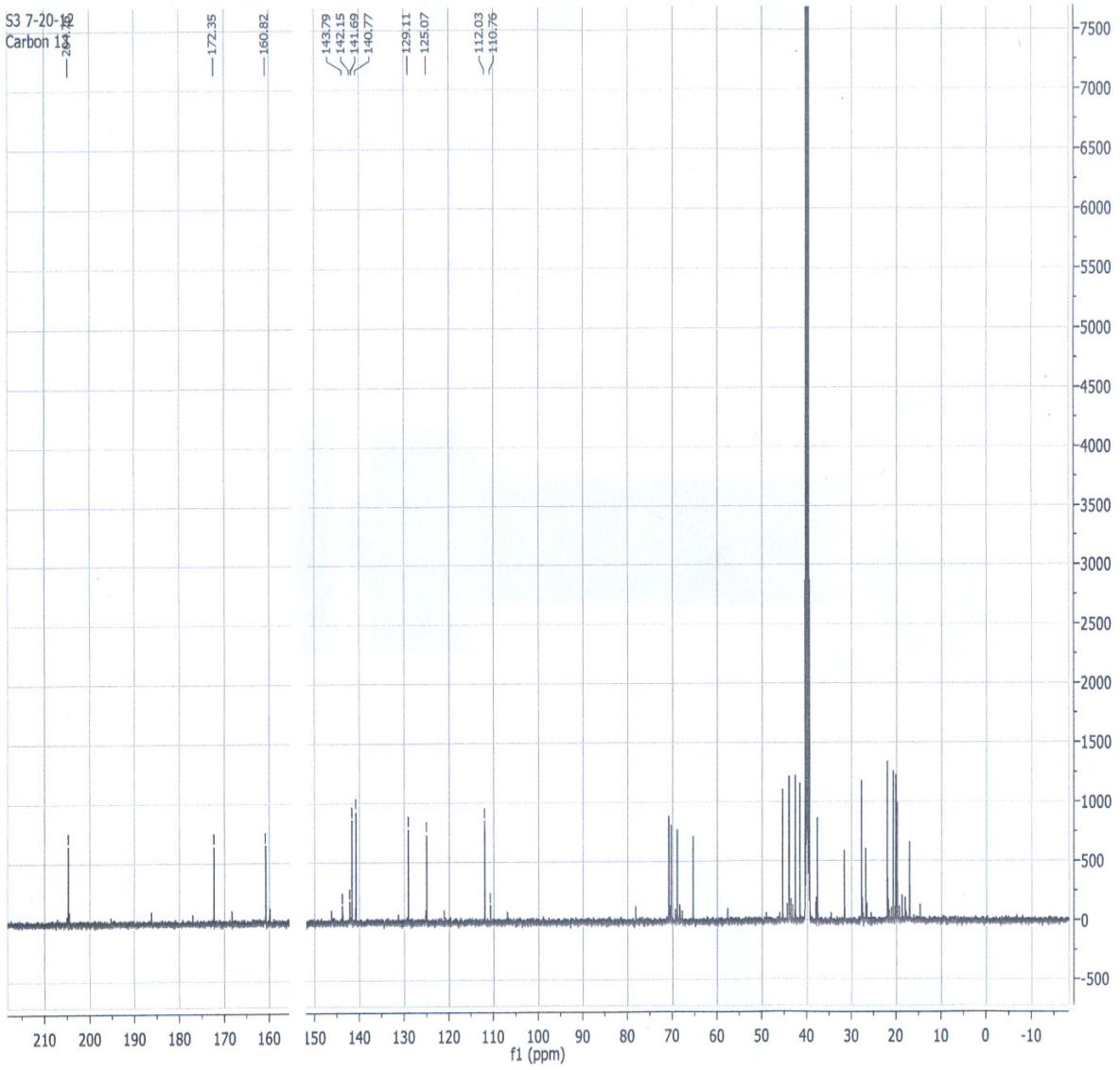

Figure 3. Full ^{13}C NMR Spectra of 7-deacetoxy-7α-hydroxygedunin potassium salt.

A detailed spectroscopic study of 7-deacetoxy-7α-hydroxygedunin potassium salt was not pursued because it is a known compound and the ^{1}H and ^{13}C NMR spectral data and meting point characteristics are consistent with that reported (Okogun and Orishadipe, 1996) for gedunin potassium salt (7).

Comparative antibacterial activities of gedunin and 7-deacetoxy-7α-hydroxygedunin potassium salt

Gedunin did not inhibit growth of all the four bacteria tested at 2000 µg/ml. On the other hand, 7-deacetoxy-7α-hydroxygedunin potassium salt had MIC of 1000 µg/ml against *K. pneumoniae,* 2000 µg/ml against *S. aureus*, and *E. coli*. However, *B. subtilis* was not susceptible to 7-deacetoxy-7α-hydroxygedunin potassium salt at 2000 µg/ml.

The standard drug Ciprotab had MIC of 488, 977, 3906 and 3906 ng/ml against *B. subtilis, S. aureus, K. pneumoniae and E. coli*, respectively (Table 1).

Characteristic structural difference between gedunin and 7-deacetoxy-7α-hydroxygedunin potassium salt are essentially in the substituent at C-7 and the nature of lactone ring. The opened lactone ring made 7-deacetoxy-7α-hydroxygedunin potassium salt very soluble in water compared to gedunin. The 7-OH group combined with the opened lactone ring make 7-deacetoxy-7α-hydroxygedunin potassium salt very polar compared to gedunin. The increased polarity and hence high solubility of 7-deacetoxy-7α-hydroxygedunin potassium salt in water most likely enhanced its observed antibacterial

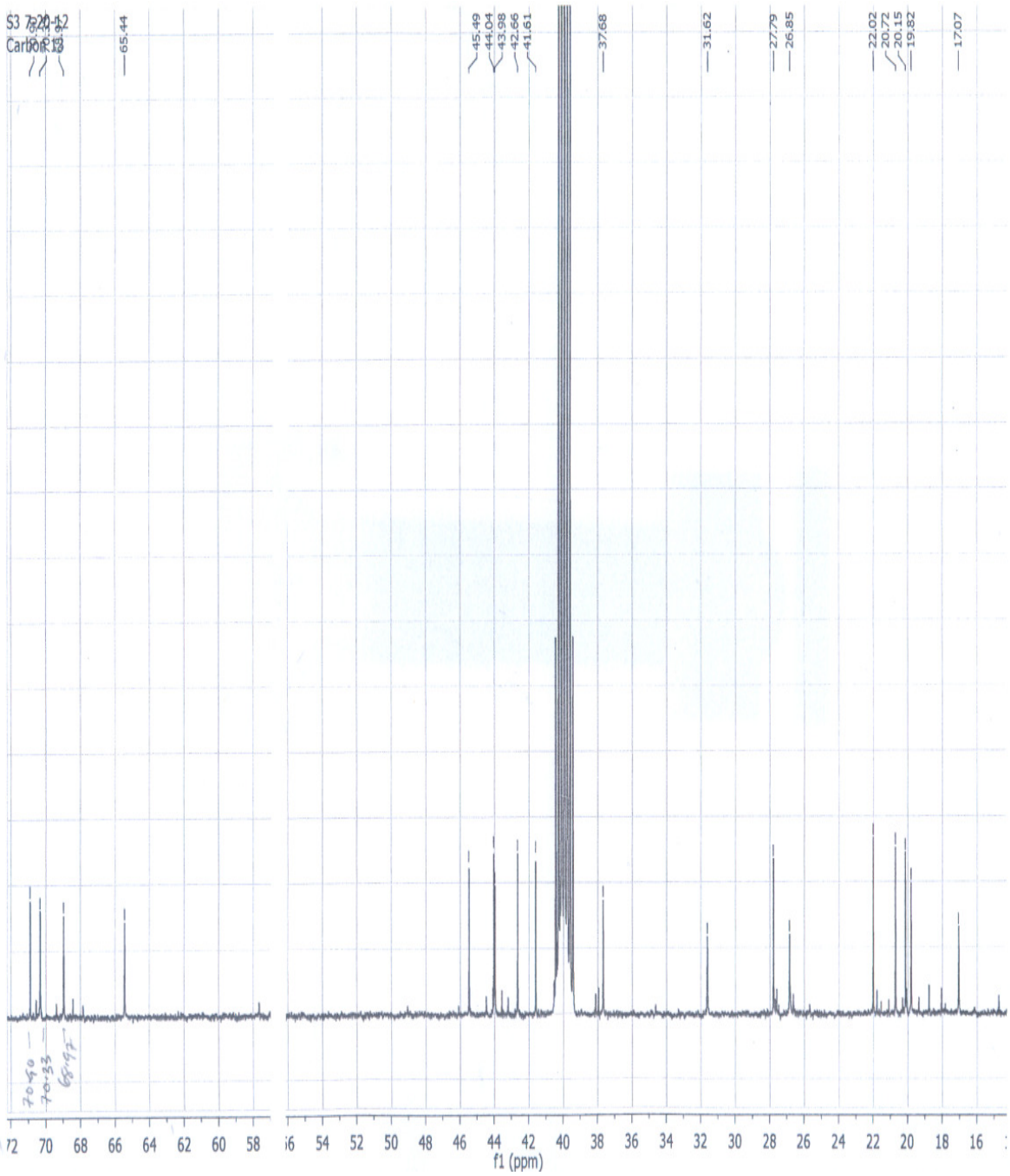

Figure 4. Expanded ^{13}C NMR spectra of 7-deacetoxy-7α-hydroxygedunin potassium salt.

Table 1. Results of antibacterial MIC for gedunin and 7-deacetoxy-7α-hydroxygedunin potassium salt.

Organism/compound	Bs	Sa	Kp	Ec
Gedunin	NA	NA	NA	NA
7-deacetoxy-7α-hydroxygedunin potassium salt	NA	2000 µg/ml	1000 µg/ml	2000 µg/ml
Organism control	NA	NA	NA	NA
Ciprotab	488 ng/ml	977 ng/ml	3906 ng/ml	3906 ng/ml

NA, No activity observed; Bs, *Bacillus subtilis*; Sa, *Staphylococcus aureus*; Kp, *Klebsiella pneumonia*; Ec, *Escherichia coli*.

activities over gedunin as it would definitely permeate the cell wall of the organisms better.

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