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Isolation and Structural Elucidation of a Novel Heptaoxygenated Xanthone from *Securidaca longepedunculata* Fresen. with Potential Antimicrobial Properties

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Abstract

In the search for novel antimicrobial agents to combat drug-resistant pathogens, we isolated and characterized a new heptaoxygenated xanthone, 1,6-dihydroxy-2,3,5,7,8-pentamethoxyxanthone (**1**), from *Securidaca longepedunculata*. The structure was elucidated using comprehensive spectroscopic techniques (IR, UV, EIMS, and 1D/2D NMR), revealing a unique substitution pattern. Additionally, the known benzophenone (4-methoxybenzo[1,3]dioxol-5-yl)-phenylmethanone (**2**) was identified. Compound **1**'s structural features suggest potential antimicrobial activity, warranting further biological evaluation. This study underscores the plant's role as a source of bioactive xanthones for addressing antimicrobial resistance.

Keywords: 1, 6-Dihydroxyl-2, 3, 5, 7, 8-pentamethoxyxanthone, *Securidaca longepedunculata*, Antimicrobial agents, xanthone, drug resistance.

Introduction

Many common infectious diseases have become difficult to treat due to drug resistance of pathogenic microorganisms. This may be due to drug overuse or the microbes developing themselves strains that can resist hitherto drugs that were previously effective in treating diseases cause by pathogenic microorganisms, which include bacteria, fungi, viruses and so on [1,2,3]. In addition, it has been reported that the average life span of an effective antibiotic is limited meaning that it becomes unstable within a very short period. This instability, also contributes to the ineffective nature of antibiotics in the treatment of pathogenic microorganisms [4].

Most of this difficult to treat diseases have been implicated to cause nosocomial infections, which are infections obtained by patients in hospital environment. The pathogenic bacteria that cause nosocomial infections include methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Klebisellia pneumoniae* [4,5]

Therefore, to remedy this problem of antibiotics resistance that emanates due to many drugs ineffective in killing of pathogenic bacteria, fungi and so on, people in both developed and developing countries are now not only embracing herbal medicine but are using it as effective drugs for their primary healthcare needs [6]. The plants used in herbal medicines for treatment of diseases caused by pathogenic microorganisms are being investigated randomly, sourcing for bioactive compounds keeping a certain biological effect in mind or through the easiest route, which involves investigating plants based on their folkloric use. Consequently, bioactive compounds, which are secondary metabolites derived from plants have longer lifespan that is fairly stable and in certain cases are more active compared to antibiotics [7].

Based on the foregoing background development, *Securidaca longepedunculata* is a medicinal plant that have been used for the treatment of many diseases. This plant is called Uwar maguguna by the Hausa speaking people in Nigeria and other countries in tropical West Africa. The literal meaning of Uwar maguguna is "mother of all medicines". It is used traditionally to treat ailments like headaches, rheumatism, diabetes, cancer, tuberculosis, veneral diseases, abortifacients and so on. The plant parts mainly used to treat diseases are the roots, stem bark or leaves [8].

Wedajo et al. [9] mentioned that the plant is a good source of bioactive secondary metabolites including alkaloids, xanthones, steroids, flavonoids and benzoic acid derivatives, which have been reported to possess antimicrobial, antimalarial, and cytotoxic activities. Among these secondary metabolites xanthones have been shown to be highly effective against pathogenic microorganisms including multidrug-resistant bacteria like MRSA and *Pseudomonas aeruginosa* etc. because of its rigid dibenzo-γ-pyrone ring structure, which confers it with high stability and due to its nature as amphiphilic compounds [12]. Consequently, in our quest to search for highly effective secondary metabolites that will help to combat the menace of antimicrobial resistance in the treatment of diseases, we report the isolation and characterization of a new heptaoxygenated xanthone together with a known compound from *Securidaca longepedunculata*.

Materials and Methods

General

UV: Shimadzu 2550 UV-visible spectrophotometer. IR: Nicolet 100 FT-IR spectrometer (Thermos scientific). GC-MS: Agilent Gas Chromatography (6890 N model) coupled to 5973 N Mass Selective Detector. NMR: Bruker Avance 400 MHz spectrometer in $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) were expressed in ppm with reference to TMS signals. DEPT-90, DEPT-135 and 2D experiments such as COSY, HSQC, and HMBC were performed using standard Bruker pulse sequence. The Bruker TopSpin software package was used for offline NMR data processing. Melting point: uncorrected with Gallenkamp apparatus.

Collection of plant

Securidaca longepedunculata roots were dug out from the soil near Kufena Mountain, Zaria – Nigeria in October, 2010. The GPS location of the sample collection site is Latitude 11°08'21.60"N and Longitude 7°39'26.67"E. The plant was identified by Mallam Mohammed Sule Gallah of the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria – Nigeria by comparison with a voucher specimen number 900148 of the plant deposited in the herbarium unit. The bark of the roots was peeled off with a table knife and debris removed with a small brush and then air – dried for about 4 days. The root bark was grinded with a pestle and mortar into coarse powder and stored in the dark with a big air – tight glass container until when used.

Extraction and isolation

The coarse powdered root bark of *Securidaca longepedunculata* (1 kg) was extracted with (4L x 3) methanol at ambient temperature for a total of 12 days. Meaning 4 L of methanol was used to carry out extraction for 4 days and the filtrate set aside in an amber colored container tightly closed. This process was repeated twice and then the three separate extract (filtrate) were combined and concentrated in vacuum with a rotary evaporator at 40 ^{o}C. The methanol extract (190 g) of the root bark was defatted by soaking it in n-hexane (500 mL x 4) at ambient temperature and allowed to stand for 2 days extraction each. The extracts (filtrate) were combined to give n-hexane fraction that was concentrated under vacuum with a rotary evaporator.

The air dried residue from n-hexane solvent fractionation was further fractionated with (500 mL x 4) each of chloroform and ethyl acetate, in the same manner as described for n-hexane fraction. The air dried residue from the preceding solvent fractionation is used for the next solvent to ensure very good extraction efficiency. The bioactive $CHCl_3$ extract was subjected to vacuum liquid chromatography (VLC) eluted with gradient n-hexane-EtOAc (100:0 – 30:70) to yield 37 fractions. Fraction 3 eluted with n-hexane-EtOAc (98:2) and Fraction 4 eluted with n-hexane-EtOAc (96:4) was combined to yield Fraction A (781.1 mg), which was further purified using VLC eluted with gradient n-hexane-EtOAc (100:0 – 30:70) to yield 21 fractions. Subfractions 4 (420.4 mg) eluted with n-hexane-EtOAc (94:6) was further subjected to VLC, eluted with gradient n-hexane-Dichloromethane (DCM) (100:0 – 5:95) to yield 20 fractions. Additional 6 fractions were obtained by further elution with DCM only to give total of 26 fractions.

Based on the TLC profile analysis Fractions 19 – 22 were combined to give pure compound **1** (13.9 mg), which was labeled AK-2, Similarly, subfraction 6 (72.8 mg) was eluted with n-hexane-EtOAc (90:10), that was obtained from fractionation of Fraction A (781.1 mg), was subsequently (subfraction 6) subjected to VLC elution with DCM only to yield Fractions 1 – 13 and further gradient elution with DCM-Me_2CO

(99:1 – 0:100) to yield fractions 14 – 23. Based on TLC profile analysis Fractions 4 and 5 were combined to give pure compound **2** (8.4 mg), which was labeled as AK-1.

1,6-Dihydroxyl-2,3,5,7,8-pentamethoxylxanthone (**1**). Pale yellow crystals, m.p. 180-182 ^{o}C; UV (MeOH) λ_{max}: 321 nm. UV (MeOH+NaOAc) λ_{max}: 373 nm. IR bands (KBr) υ_{max}: 3423, 2940, 1650, 1592, 1464, 1423, 1059, 915, 813, 670 cm^{-1}. LREIMS: 378.1 [M^{+}], 363, 333, 305, 249, 229, 205, 174, 153, 125, 93. ^{1}H and ^{13}C NMR (Table 1).

Results and Discussion

Compound **1** was isolated as pale yellow crystals with melting point 180-182 ^{o}C. Its molecular formula was deduced as $C_{18}H_{18}O_9$ based on the information obtained from the low resolution electron impact mass spectrometry (LREIMS), its molecular ion *m/z* 378 [M] $^{+}$, as shown in its mass spectrum (Figure 1) The compound has double bond equivalent (DBE) of 10, meaning that it has 8 double bonds accounted for in two benzene rings, one double bond equivalent to the carbonyl group and the remaining one double bond equivalent to the γ-pyrone ring. This structural composition is consistent with the compound being a xanthone, in line with the basic skeleton of xanthone shown in Figure 2 [10,11,12].

The ^{1}H NMR spectra of compound **1** (Figure 3) indicated the presence of five methoxyl group signals at δ_H 3.92 (s, 3H), 3.91 (s, 3H), 3.98 (s, 3H), 4.09 (s, 3H) and 3.95 (s, 3H). Also, one singlet of aromatic proton at δ_H 6.35 assignable to H-4 of the xanthone A ring. There was a sharp singlet of hydroxyl proton at δ_H 13.15 assignable to 1-OH, which indicated the existence of intramolecular hydrogen bond. The presence of this peak at δ_H 13.15, indicated that the hydroxyl group was chelated, being *peri* to the carbonyl function, i.e. at C-1 or C-8. Another, hydroxyl proton with a low intensity broad singlet resonating at δ_H 6.42, was assignable to 6-OH in the xanthone B ring. These peak assignments were matched with the corresponding proton in the structure of **1** as shown in Table 1.

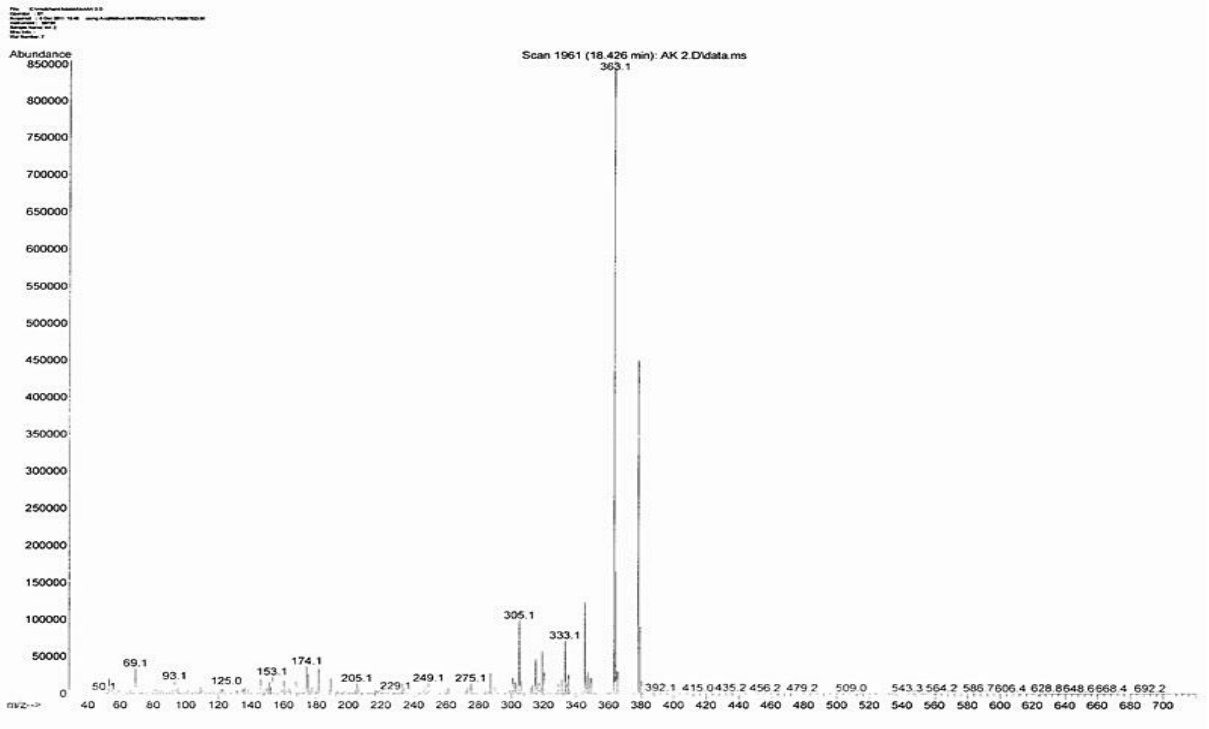
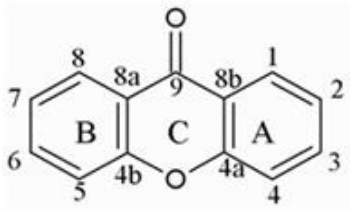
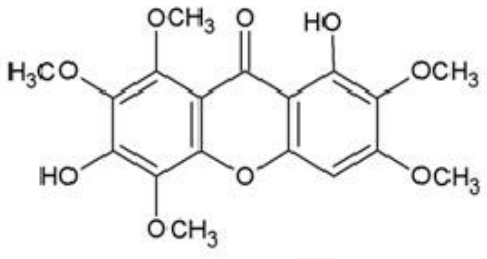


Figure 1: Mass spectrum of compound **1**



Basic skeleton of xanthone



1

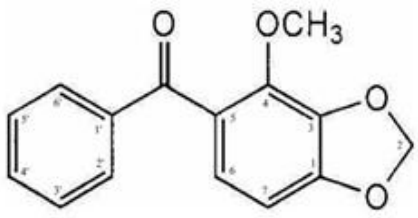


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Figure 2: Basic skeleton of xanthone and structure of compounds 1 and 2.

The broadband decoupled ^{13}C NMR spectrum of compound **1** (Figure 4) showed eighteen (18) carbon peaks. The multiplicities were determined by DEPT experiments using the pulse angles suggested by [13] and the result is shown in Figure 5 for accurate and easy assignment of the peaks in the ^{13}C NMR spectrum. The DEPT-135 indicated peaks due to one methine and five methyl carbons as positive, while there is no methylene peak. DEPT-90 indicated the presence of one methine carbon peak and DEPT-45 indicated that there are twelve quaternary carbon atoms, since they are the peaks in the ^{13}C NMR spectrum that did not appear in the DEPT-45 spectrum. Each carbon atom in **1** was assigned by using these results and comparison with other polyoxygenated xanthones reported in the literature. These assignments revealed the presence of one carbonyl carbon (δ_C 180.52), five methyl, one methine and twelve quaternary sp^2 carbons. The methine peak at δ_C 94.88 was assigned to C-4, while the five methyl signals are highly deshielded, which is an indication that they are bond to a highly electronegative atom, specifically oxygen, resonated at δ_C 56.32, 61.54, 61.71, 61.74 and 62.13 for the carbon of methoxyl groups at C-3, C-2, C-7, C-5 and C-8 respectively. Table 1, shows the peak assignments for each carbon atom present in the structure of **1** based on the above mentioned assignment utilizing the peaks in the ^{13}C NMR spectrum (Figure 4).

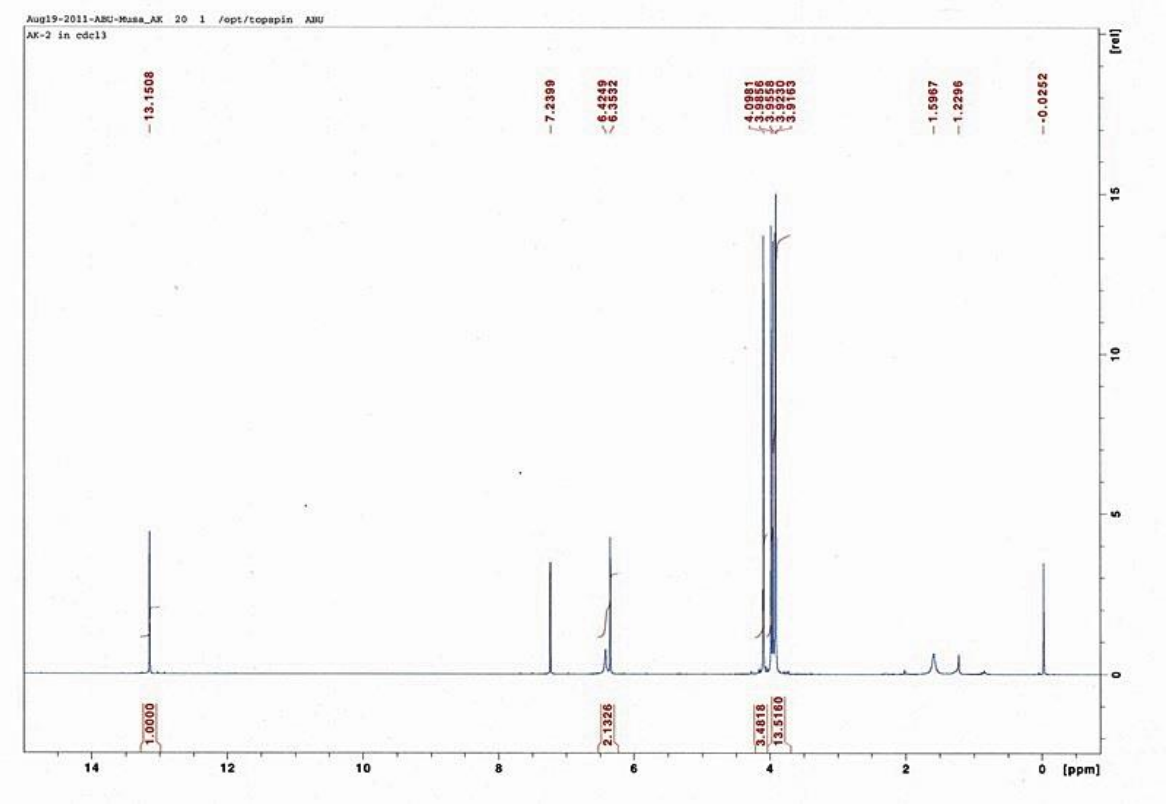

Figure 3: ^{1}H NMR spectrum of compound **1**

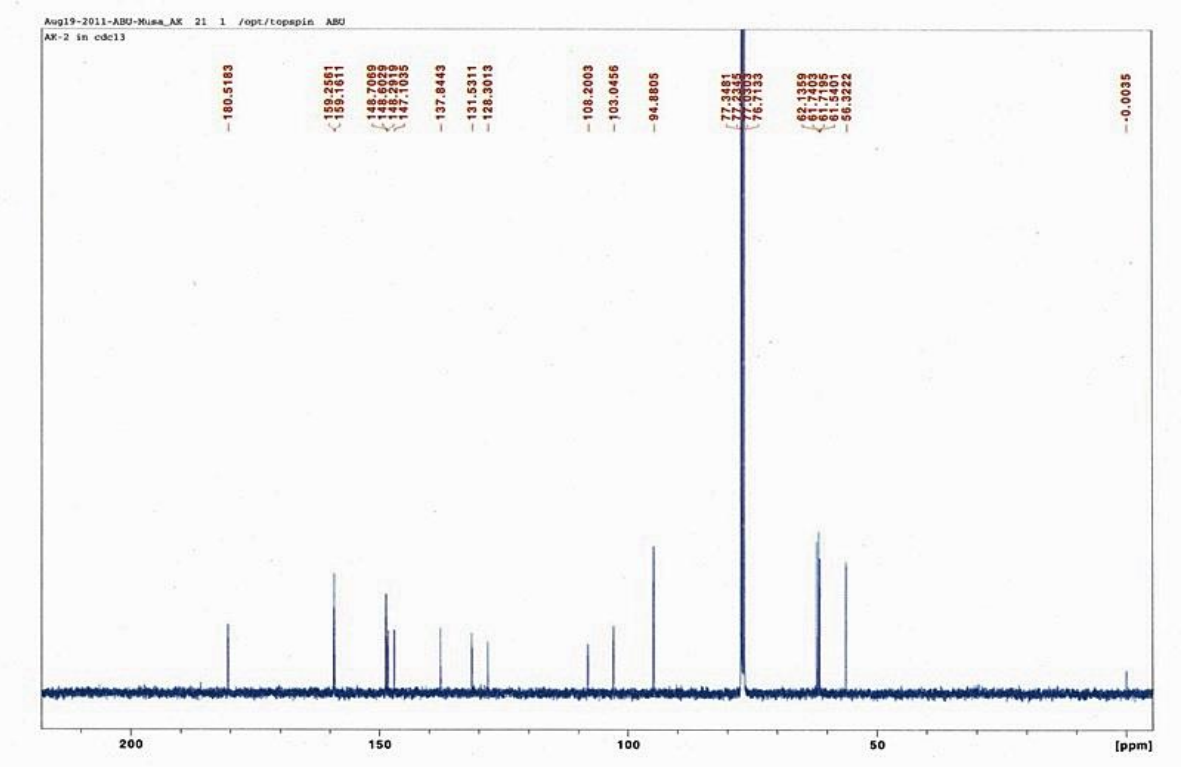

Figure 4: ^{13}C NMR spectrum of compound **1**

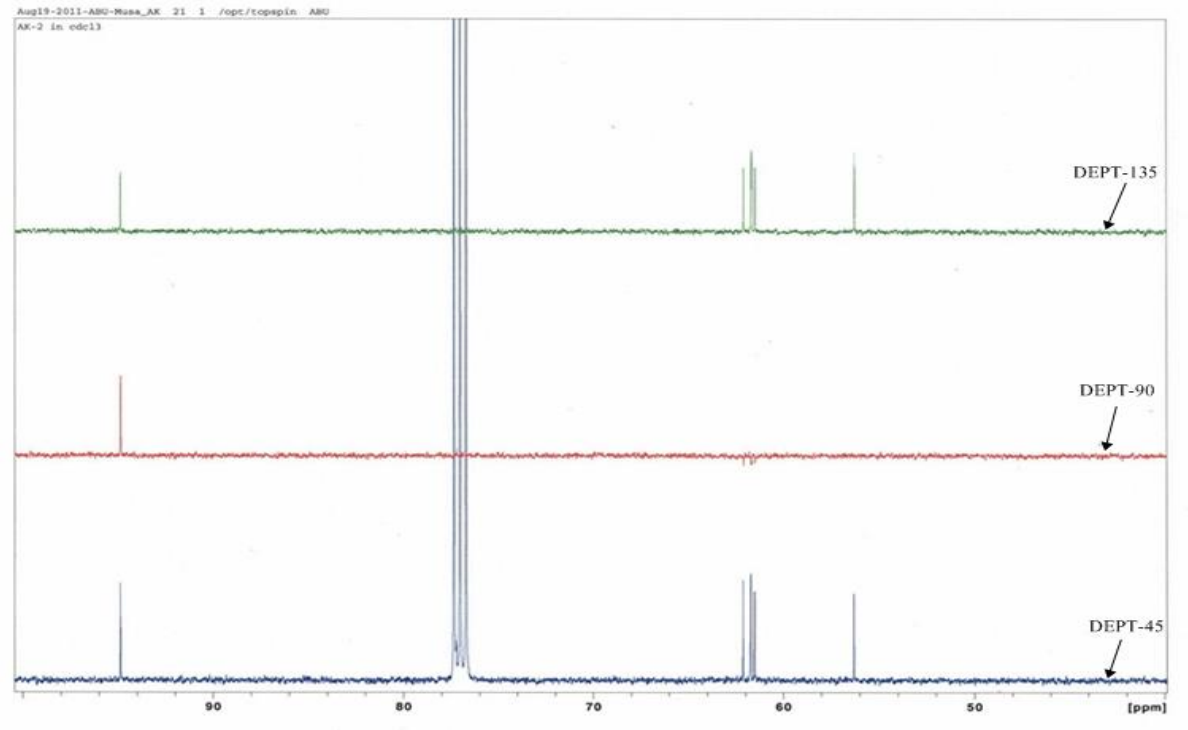

Figure 5: DEPT spectrum of compound 1

The exact position of each methoxyl group was established by HSQC experiments. The results of the HSQC experiment on **1** are shown in Figure 6. The HSQC spectrum of compound **1** showed that the methoxyl group carbon resonating at δ_C 61.54 (C-2) was coupled with the proton at δ_H 3.92, also the methoxyl group carbon resonating at δ_C 56.32 (C-3) showed a cross peak with the proton at δ_H 3.91. Similarly, the carbon of methoxyl group at δ_C 61.74 (C-5) was coupled with the proton at δ_H 3.98, while the methoxyl group carbon resonating at δ_C 61.71 (C-7) showed a cross peak with the proton at δ_H 4.09. In addition, the methoxyl group carbon at δ_C 62.13 (C-8) was coupled with the proton at δ_H 3.95.

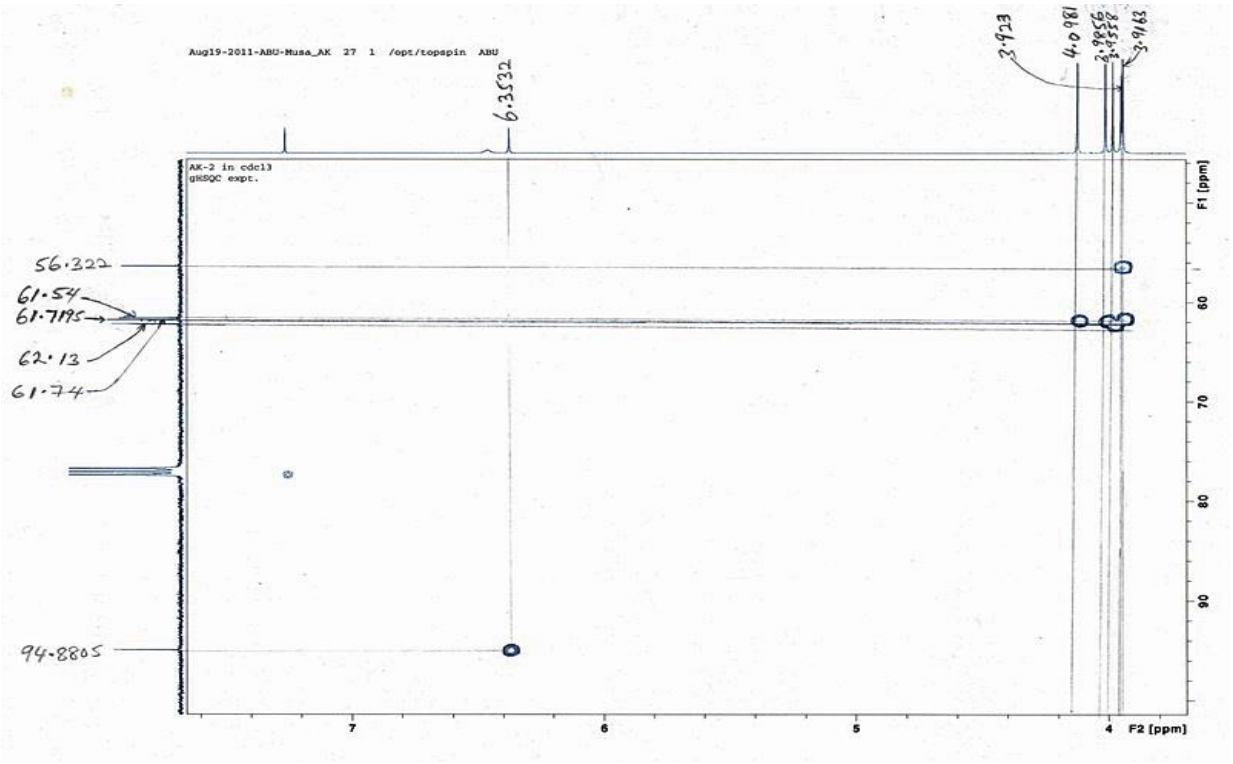

Figure 6: HSQC spectrum of compound 1

Consideration of the results obtained from the ^{13}C NMR (Figure 4), DEPT (Figure 5) and 2D HSQC experiments (Figure 6) indicated that ring A of the isolated xanthone (**1**) contains a chelated hydroxyl group at C-1 and ring B contains a free hydroxyl group at C-6. In addition, two methoxyl groups are present at C-2 and C-3 in ring A, while three methoxyl groups were present in ring B at C-5, C-7 and C-8. These assignments were further confirmed by the long range proton-carbon connectivity from HMBC spectrum.

The HMBC spectrum results are shown in Figure 7, which indicated that the proton at δ_H 6.35 was coupled with the carbons at δ_C 128.30 (C-2), 159.25 (C-3), 159.16 (C-4a), and 103.04 (C-8b).

Therefore, this signal was unambiguously assigned to H-4. The sharp proton singlet at δ_H 13.15 assignable to 1-OH was coupled with the carbons resonating at δ_C 159.25 (C-3), 94.88 (C-4), and 103.04 (C-8b). These correlations suggested the location of this hydroxyl group at C-1

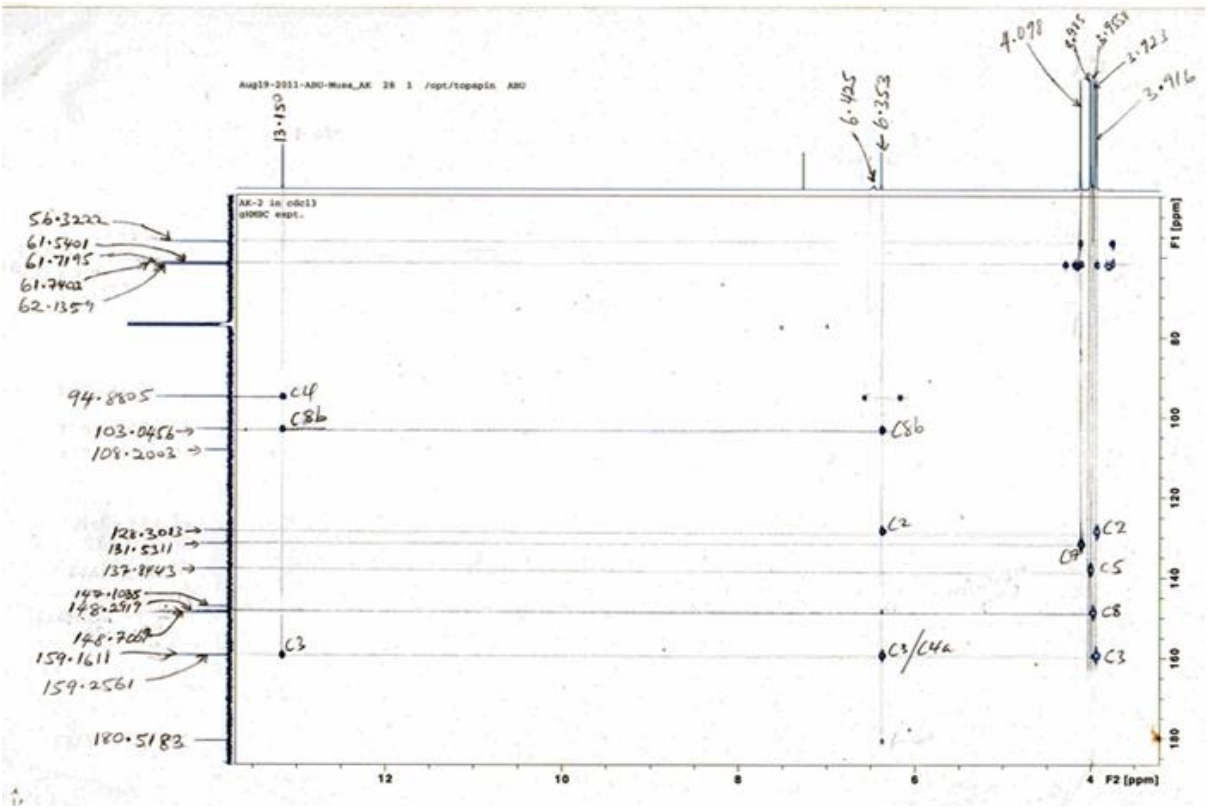

Figure 7: HMBC spectrum of compound **1**

The positions of the five methoxyl groups were established by HMBC experiment. Two methoxyl groups are placed in the A ring, because the HMBC spectrum (Figure 7) indicated that the proton singlet at δ_H 3.92 showed a cross peak with C-2 (δ_C 128.30), while the one at δ_H 3.91 showed a cross peak with C-3 (δ_C 159.25). The remaining three methoxyl groups were placed in the B pyran ring, due to the fact that the HMBC spectrum showed that the proton singlet resonating at δ_H 3.98 showed a cross peak with C-5 (δ_C 137.84). Likewise, the proton resonating at δ_H 4.09 showed a cross peak with C-7 (δ_C 131.53), and the proton singlet at δ_H 3.95 showed a cross peak with C-8 (δ_C 148.29). These complete HMBC correlation assignments are shown in Figure 8, which indicated some of the significant correlations in the structure of **1**. Table 1, shows the full assignments of the peaks in the ^{1}H and ^{13}C NMR spectra, with the aid of DEPT, HSQC and HMBC experiments.

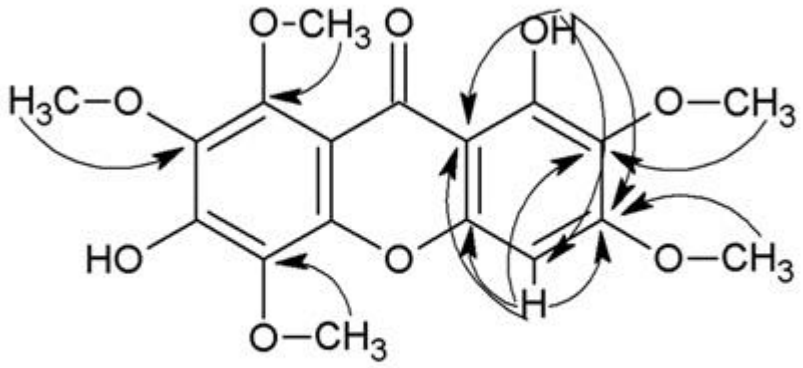

Figure 8. All HMBC correlations (⟶) for compound **1**

The FT-IR spectra data are in line with reported values in the literature, which suggested that compound **1** is a xanthone [14,15]. Furthermore, the UV absorption maximum at 321 nm (Figure 9) is typical of the values obtained for a xanthone nucleus [16,17]. However, the shift of the absorption maximum to 373 nm (Figure 10), after addition of sodium acetate indicated the presence of a free hydroxyl group at position C-3 or C- 6 on the xanthone [15,18].

The EIMS spectrum (Figure 1) revealed that **1** is a pentamethyl substituted xanthone because most of the fragment ions produced possesses the loss of five methyl groups ($5CH_3$). Smith [19] reported that the loss of HCCH group can only take place, if a compound is aromatic in nature, especially benzene and its derivatives. The loss of HCCH group is evident of the formation of fragment ions *m/z* 249, 205, 174, 69 and 50. The fragment ions 69 and 50 shows that a carbonyl group is present in compound **1**. The entire above mentioned fragment ions produced from the EIMS of compound **1** suggested that it is a substituted [20]. Its detailed substitution pattern was revealed by NMR spectroscopic experiments that are discussed below.

Table 1: ^{1}H and ^{13}C NMR data of **1** (400 and 100 MHz, $CDCl_3$, *J* in Hz, δ in ppm)

Position	DEPT	δ_C	δ_H	HMBC
1	C	148.08		
2	C	128.30		
3	C	159.25		
4	CH	94.88	6.35,s,1H	C-2,C-3,C-4a,C-8b
4a	C	159.16		
4b	C	147.10		
5	C	137.84		
6	C	148.60		
7	C	131.53		
8	C	148.29		
8a	C	108.20		
8b	C	103.05		
9	C	180.52		
2-OCH_3		61.54	3.92,s,3H	C-2
3- OCH_3		56.32	3.91,s,3H	C-3
5- OCH_3		61.74	3.98,s,3H	C-5
7- OCH_3		61.72	4.09,s,3H	C-7
8- OCH_3		62.13	3.95,s,3H	C-8
1-OH			13.15,s,3H	C-3,C-4,C-8b
6-OH			6.42,br s,3H	

Key: s = singlet, br s = broad singlet

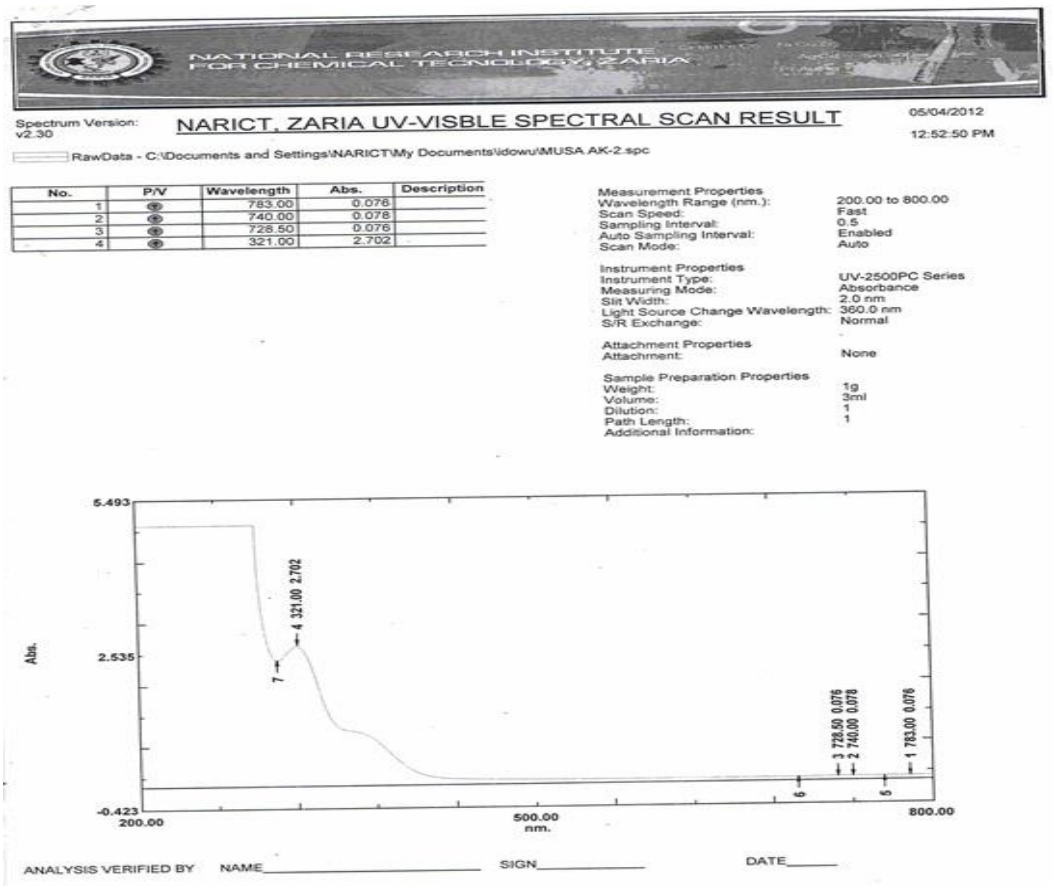

Figure 9: UV-VIS spectrum of compound **1** in MeOH

The sharp peak at δ_H 13.15 in the ^{1}H NMR spectrum, which indicated the existence of intramolecular hydrogen bond is in line with similarly reported peak values in the literature, which places it at 1-OH [21]. This peak value implies that the hydroxyl group was chelated, being *peri* to the carbonyl function, i.e. at C-1 or C-8 [14]. The remaining, hydroxyl proton with a low intensity broad singlet resonating at δ_H 6.42, was assignable to 6-OH. This assignment is supported by the UV spectral data as explained above. An isolated proton peak at δ_H 6.35 is assigned to H-4. These three ^{1}H NMR peaks for 1-OH, H-4 and 6-OH are in agreement with the reported peak assignment for 1, 6-Dihydroxy-3, 7-dimethoxy-2-(3-methyl-but-2-enyl)-xanthone [14].

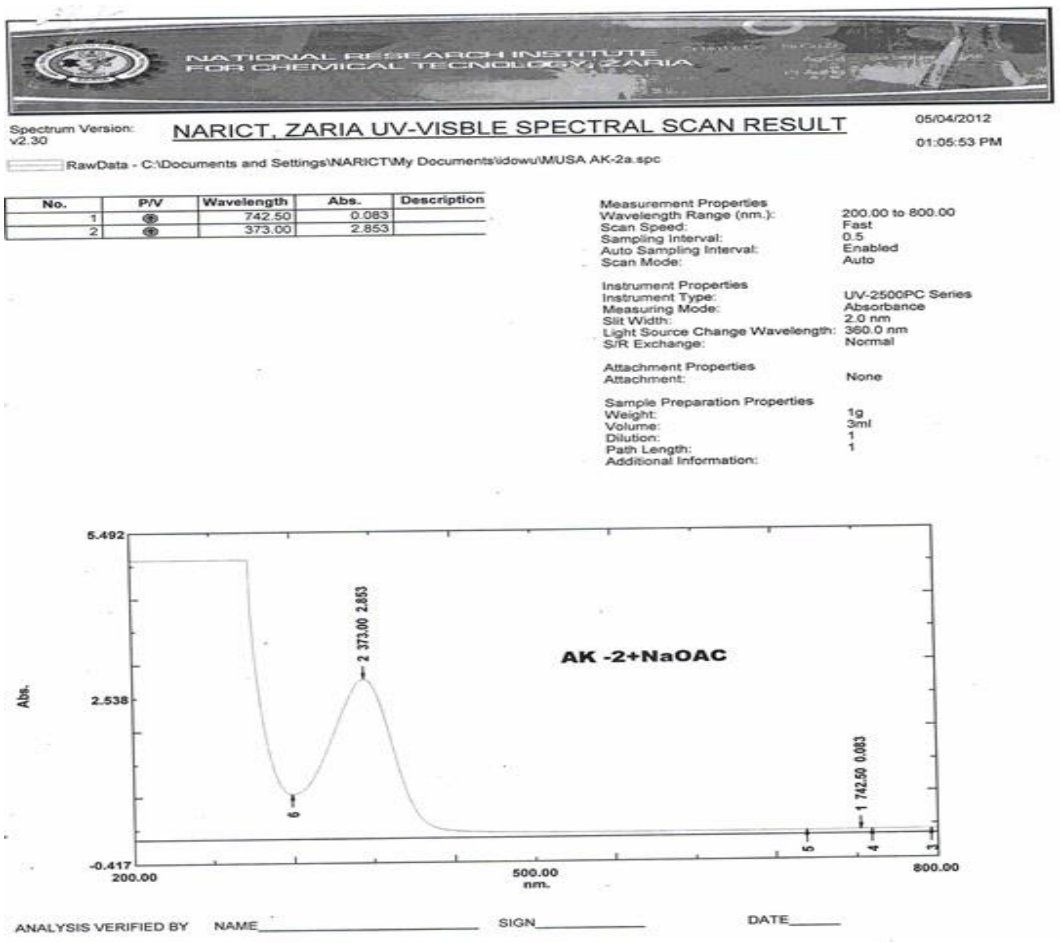

Figure 10: UV-VIS spectrum of compound **1** in MeOH+NaOAc

The ^{13}C NMR spectrum of compound **1** has two aromatic rings, because it showed the characteristics signals of two aromatic rings resonating at δ_C 137.8 and 128.30. This is in agreement with reported values in the literature [22]. Furthermore, the HMBC correlations of **1** that established the position of H-4 is similar to the one reported for the same H-4 position in the A ring of swertiadecoraxanthone II, known as 1, 3, 8-trihydroxy-2, 5-dimethoxyxanthone [23]. The position of 1-OH was placed at C-1 based on its HMBC correlations with C-3, C-4 and C-8b. It is important to point out that the hydroxyl proton at C-1 rarely shows cross peak with carbon at C-3. However, Marti *et al.* [24] reported its existence in the HMBC correlation for 1, 5, 6-Trihydroxyl-3-methoxy-7-geranylxanthone.

Also, Cheng *et al.* [25] demonstrated its presence in the HMBC correlation for the compound Calophinone.

The above ^{1}H and ^{13}C NMR spectra peak assignments together with the UV spectral data assignments, which firmly established the position of the free hydroxyl (–OH) at C-6, gives rise to the unambiguous structure of compound **1** as

1, 6-Dihydroxyl-2, 3, 5, 7, 8-pentamethoxyxanthone, shown in Figure 2.

It is important to point out that there is an isomer of compound **1**, which have been isolated from this same plant, which include 1, 5-Dihdyroxyl-2, 3, 6, 7, 8-pentamethoxyxanthone, known by the trivial name Securidacaxanthone A [26], because both compounds have the same molecular mass as 378 $[M]^+$. However, they differ in their structural formulae due to the location of the free hydroxyl group.

Compound **2** was isolated as light yellow oil. Its mass spectrum and ^{1}H NMR spectra data are in strong agreement with similar values of a previously isolated benzophenone reported in the literature known as (4-methoxybenzo[1, 3]dioxol-5-yl)-phenylmethanone as shown in Figure 1 [27].

Conclusion

The isolated xanthone (**1**) represents a new addition to the heptaoxygenated xanthone family, with a substitution pattern distinct from known analogs like Securidacaxanthone A. Future studies should evaluate its activity against WHO-priority pathogens (e.g., MRSA, *P. aeruginosa*) using standardized MIC assays. Additionally, cytotoxicity screening against cancer cell lines (e.g., MCF-7, HeLa) could reveal therapeutic potential.

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