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Research Paper

Tetrahydroanthracene Derivative: Anti-microbial Isolate from Acanthospermum hispidum DC

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Abstract

The leaves and stems of Acanthospermum hispidium were extracted with distilled ethanol using cold extraction and concentrated using a rotary evaporator at 37 ^{0}C. The crude extract was partitioned successively using hexane, benzene and methanol. Fractions 19, 20 and 21 purified on Sephadex LH-20 gave a compound elucidated to be 1, 3, 6, 8-tetrahydroxyl-9-anthracene carbonaldehyde, using the state-of-art tools of spectrometry. The results of the antimicrobial test on the isolated compound show activity against P. mirabilis, B. subtilis, P. aeruginosa, C. albican, S. typhi and B. cereus at minimum inhibitory concentration (MIC) value of 100ppm.

Keywords: *Acanthospermum hispidum, Anthracene Carbonaldehyde, Antimicrobial Test, Minimum Inhibitory Concentration.*

1. Introduction

Ancanthospermum hispidum DC (Compositae) is a medicinal plant commonly known as 'ewe onitan meta' in western Nigeria. The plant is a bushy, annual of about 50 cm high, commonly found in a waste places or cultivated land. The cultivated specimens of Acanthospermum hispidium will germinate on a wide variety of soils, from sandy to clay. The transition from the flowering to the fruiting phase of this species is extremely rapid, demonstrating a metabolic priority of reproduction over the elaboration of chemical defences (Miranda, 1996). The leaves are used locally for the treatment of acute tuberculosis, cough, diarrhoea, dysentery, typhoid and pneumonia (Burkill, 1985). In Ghana, the leaves are used to cure kpiti (leprosy). In Congo the plant is used to treat stomach complaints, wounds and migraine. It is used throughout north eastern Brazil as a folk medicine for asthma (Evani' de, 2008).

Traces of alkaloid have been reported in the whole plant and in the leaves (Burkill, 1985). The ethanol extract of the leaves and flowering tops of Acanthospermum hispidum was showed to have varying degrees of activity against wide range of pathogenic bacteria but no activity was observed for the aqueous extract of the fresh plant material (Fleisher et al, 2003). The effects of administering Acanthospermum hispidum DC ethanolic leaf extract on patients of hepatitis were studied on acute hepatitis induced by carbon tetrachloride in rats (Edewor and Olajire, 2007). The effects were monitored by estimating the serum transaminases levels and the histopathological changes in the livers of experimental rats. The pre-treatment of the animals with Ancanthospermum hispidum DC leaf extract (0.3-2.0 g kg-1) significantly elevated the activities of the serum transaminases as well as the hepathotoxin-induced histopathological changes in the livers of experimental rats (Edewor and Olajire, 2007). Some of the compounds that have been isolated from the plant are acanthamolide, acanthoaustralide, germacratrienolide and it derivatives and loliolide (Antagera, 2000). In this paper, we report for the first time the anti-bacterial and cytotoxicity of the extracts obtained from the leaves and stems of Ancanthospermum hispidum DC and the newly identified compound, using the state-of-art-tools spectrometry.

2. Materials and methods

General: Column chromatography: silica-gel (merck, 60-200 mesh) Sephadex^{TM} LH-20 (GE health care Bio-science AB). IR spectra: FTIR Nicolet Avater 330, thermo-electron operation. ^{1}H and ^{13}C NMR mecury-200BB. Mass spectra: Fainnigan 4000 spectrometer (low resolution) krats 50 spectrometer (high resolution).

2.1. Plant materials

The leaves and stems of Acanthospermum hispidum DC were collected from the Department of Botany, University of Ibadan. The plant was identified by Mr Donatus Esimakhuai of the Herbarium section of the Department of Botany, University of Ibadan. It was later identified and authenticated by Dr. Ayodele of the same department, with Herbarium No UIH22301.

2.2. Extraction and isolation

Air dried leaves and stems (800 g) were milled into powder with the aid of an electrical grinder. Distilled ethanol (6 L) was added to the mixture of the plant in a stopper glass container. It was left for one week and then finally filtered. The ethanol extract obtained was concentrated at 37^{0}C using rotary evaporator, at reduced pressure. It was then fractionated with n-hexane and methanol. The methanol extract was later macerated with benzene and were separately concentrated with rotary evaporator to give the refine-methanol and benzene extract respectively. The benzene extract was subjected to open column packed with silica gel (70-220 mesh size) using a dry method. The column was eluted with a gradient of n-hexane/ethyl acetate. A total of 27 eluents were ob-

tained. Phytochemical analysis was carried out on the chromatographic fractions. The eluents 19, 20 and 21, was seen to contained alkaloids. The fractions were combined together as fraction (A) based on their TLC pattern. The mixture was concentrated using rotary evaporator. Fraction (A) was loaded on the Sephadex LH-20. The column was eluted with methanol (100%). Eluents were collected at 5 ml rate. Two types of crystals were obtained which were purified using isopropyl alcohols separately. The two crystals were further subjected to 2D TLC. The best spot was sent for spectroscopic analysis.

2.3. Anti-microbial screening

The methanol and fraction (A) extracts were screened for antimicrobial activities against 5 standard strains of bacteria and a fungus: Staphylococcus typhi ATCC 2785, Bacillus subtilis ATTC 14579, Bacillus cereus ATTC 33923, Pseudomonas aeruginosa ATTC 27856, Proteus mirabilis ATTC 21784, Candida albican MTTC 227. The antimicrobial assay was performed according to standard method (Bauer et. al, 1996) zones of inhibition was measured with frequent ruler. Determination of MIC was conducted following Bauer Method. The results are presented in Table I.

2.4. Brine shrimp lethality test (BST)

The BST was performed according to standard protocol (5, 6) and LC_{50} values in µg/ml were determined for partitioned fractions, pooled chromatographic fractions and isolated compound I. The results are presented in table II.

3. Results and discussion

The results of the phytochemical studies on the extracts of Ancanthospermum hispidum show the presence of alkaloids, phenolic compounds, resin, cardiac glycoside, saponins and tannins. This confirmed with what was reported by (Odebiyi et. al,1978 and Sanon et.al, 2003).The results of the antimicrobial test on the methanol extracts and the isolated compound I are shown in Table I. The extract shows activity against P. mirabilis, B. subtilis, P. aeruginosa, C. albican, S. typhi and B. cereus at MIC of 100 ppm. The antimicrobial activities of the methanol extracts and compound I suggested their usefulness in the treatment of infectious diseases caused by the tested microbes. BST results of the methanol extract gave LC_{50} > 1000. This value indicated that the methanol extract is non-toxic. However LC_{50} result on compound I was not converged after several attempts. Compound I (1, 3, 6, 8-tetrahhydroxy-9-anthracene carbaldehyde) was obtained as brown crystals. On purification with isopropyl alcohol, the crystals turned dirty white. The IR spectrum showed the characteristics signals of –OH (3471 cm^{-1}), conjugated carbonyl carbon (1658 cm^{-1}), C=C (1444 cm^{-1}), C-O stretching vibration (1210 cm-1) and fingertip region of aromatic benzene skeleton. The C-H stretching vibration at 2900 cm-1, is not very pronounced due to lack of aliphatic moiety. The UV spectra data of compound I was characteristics of the anthracene chromophore and the sodium acetate addition caused a bathochromic shift, indicating the presence of hydroxyl group; the consecutive addition of H_3BO_3 did not modify the UV spectrum, suggesting the absence of the ortho hydroxyls. The molecular formula was determined as $C_{15}H_{10}O_5$ on the basis of HREMS (m/z 270.115 cal. 270.314). The standard ^{13}C NMR spectrum as well as polarization transfer (DEPT) experiment of compound I depicted fifteen carbon atoms. The ^{13}C NMR (DEPT) showed resonance for nine quaternary carbons and six methines giving an attached proton formula of $C_{15}H_{6.}$ This molecular formula possesses a double bond equivalent of eleven, which was consistent with the exact mass measurements. The clustering of signals between δ 7.05 and 7.99 in ^{1}HNMR spectrum of compound 1 is a characteristic of phenolic or aromatic moiety. The presence of signal at δ 10.5 which crossed peak with δC at 171.1 is an indication of aldehydes functional group. Absent of aliphatic entity was supported due to no signal up field in the ^{1}HMNR of compound 1. The ^{1}HMNR spectrum in the aromatic region showed five protons in three different environments. The integral pattern in ^{1}HMNR depicted 2:2:1:1. The signals downfield which are not directly attached to any carbon in HSQC experiment also corroborated the presence and attachment of heteroatoms like oxygen. The coupling constant displayed by the ^{1}HMNR spectrum in all coupled protons is within 1.4 Hz suggesting Meta coupling arrangement. The connectivity within the molecule was shown by ^{1}H-^{1}H cosy and HMBC experiments. The ^{1}HMNR spectrum showed the presence of five aromatics protons at δ 7.58 (2H, d, J=1.4 Hz), δ 7.99 (1H, s) and δ 7.05 (2H, d, J=1.4 Hz). The proton at δ 9.98 suggested the presence of an aldehydes moiety. The connectivity between the proton of the aldehydes and that of the benzene moiety was done through ^{1}H-^{1}H cosy cross- peaks. The broad nature of the OH in IR spectrum also corroborates the presence of aldehydes moiety closer to OH entity. A ^{1}H-^{13}C 2D NMR shift correlated measurement (HMBC) showed long range coupling between H-17 and H-21/H-10; H-12 and H-20 which is consistent with and independently confirming the structure of compound I. Based on the spectra data, the compound is trivially named 1, 3, 6, 8-tetrahydroxy-9-anthracene carbaldehyde in figure I.

Table 1: Antimicrobial Activity of Methanol Extract

Micro- organism	Zone of inhibition (mm)	
	Methanol	Isolated compound
P.aeruginosa	12.7	15.0
P.mirabilis	12.0	13.0
B.subtilis	12.3	12.0
C.albican	9.0	14.0
S.typhi	11.0	13.3
B.cereus	9.7	14.3

Table 2: ^{1}HMNR and ^{13}C NMR of Compound I

s/n	δH	M	I.f	Δc	HMBC(H/C)
1.	-			162.4 (s)	
2.	7.05	D	1H	99.3 (d)	3/6,4/5,9a/8a,1/8
3.				149.9 (s)	
4.	7.58	D	1H	115.6 (d)	4a/10a,10,9a/8a
4a.				119.8 (s)	
5.	7.58	D	1H	115.6 (d)	4a/10a,10,9a/8a
6.				149.9 (s)	
7.	7.05	D	1H	99.3 (d)	3/6,4/5,9a/8a,1/8
8.	-			162.4 (s)	
8a.				97.1 (s)	
9.				118.6 (s)	
9a.				97.1 (s)	
10.	7.99	S	1H	131.2 (d)	4a,8a,9a,10a
10a.	9.98	Bs	1H	119.8(s)	9,9a
11.				171.1 (d)	

UV λmax ($CHCl_3$) 251 nm;
IR (Nujor): 3538 (OH); 1051 (C-O); 1600 (C=C) and 2958 (CH srt)
EIMS (low and high resolution): m/z 398 (80%) - H_2O, 317; 300; 224
CIMS m/e 190.2, 189.2, 188.1, 121.3, 69, 42.9

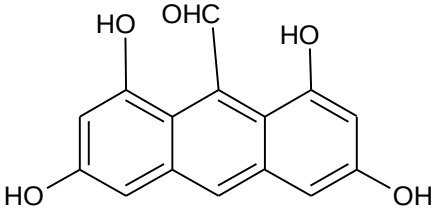

Fig. 1: Structure of Phenanthrenol

^{1}H NMR (400 MHz, $CHCl_3$): δ 1.10 (3H, t, H-30); δ 2.65 (1H, bm, H-6); δ 6.53 (1H, d, H-17); δ 4.10 (2H, m, H-18); δ 5.00 (1H, m, H-19); δ 5.01 (1H, m, H-22); δ 5.34 (1H, M, H-23)
^{13}C NMR (100 MHz, $CHCl_3$): see Table I

4. Conclusion

The structure of the compound gotten conforms to that which has been reported before and this is only a derivative of the compound found in Acanthospermum hispidum DC. The extracts of the plant has also been shown to have antimicrobial properties.

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