ISOLATION AND IDENTIFICATION OF THE CHEMICAL CONSTITUENTS OF ANTHOCLEISTA DJALONENSIS CHEV.

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Abstract

Phytochemical screening of the stem bark of Anthocleista djalonensis chev., (legoaniaceae) revealed the presence of flavonoids, which on further separation and isolation gave an amorphous crystalline compound AJEE1 400mg. The structure of this compound was established as polyarvin, a chalcone on the basis of spectra evidence, ^{13} C NMR and MS. Further proof was based on the chalcone condensation reaction followed by mixed melting point and co TLC comparison with an authentic sample.

Key words: Anthocleista djalonensis, flavonoid, polyarvin, 2D NMR, HMBC

Introduction

Anthocleista djalonensis chev. Family legoaniaceae is a tropical plant that is widely distributed in the savannah forest of West African coast, most especially Cameroun, Nigeria, Ghana, Ivory Coast and Guinea.(1). Some of the other species of this plant such as Anthocleista vogelli has been found in East African coast with some anticonvulsant activities(2).The stem bark and root of this plant are used in Nigeria and Ghana for the treatment of skin infections(3).The stems are sometimes hallowed out in Northern Nigeria for use as quivers, hence the Hausa name, Kwari.(4). The root decoction is used in Ivory Coast as a poison anti-dote for leprosy and for the treatment of oedema. (5) while in Sierra leone the root decoction is used for treatment of gonorrhea(6).In the western part of Nigeria the local traditional healers use the seeds of the fruit of this plant for abdominal pains and stomach discomfort. Previous studies on the methanol extract of the root of Anthocleista djalonensis showed some anti-microbial activities against bacillus subtilis and staphylococcus aureus(7). Apart from the report already mentioned, there has been no information on the structural elucidation and chemical constituents of the methanolic extract of this plant in the literature.

This present study has been designed to investigate and isolate the chemical constituents of the stem bark of Anthocleista djalonensis and determine their structures.

Materials and Methods:

The stem bark of the Anthocleista djalonensis were collected on Lagos/Badagry express way in the south western part of Nigeria in the months of May through June 1999 with the aid of Olatunde Aliyu of 10 Ladipo street, Mushin Lagos, a local traditional healer. The plant material was authenticated by comparison with a herbarium sample at the department of Pharmacognosy , School of Pharmacy of the College of Medicine, Lagos, Nigeria by Mr Adelelke Olusegun and Forestry Research Institute of Nigeria, Ibadan. A voucher specimen FHI 25437 is deposited at the Forestry department of the Research Institute. The fresh stem bark were dried in a hot air oven at 40°C for 5 days and thereafter milled into a fine powder and kept in a clean container and subsequently subjected to standard phytochemical procedures for the presence of steroids, alkaloids, anthraquinones, tanins and flavonoids.

Melting point were determine on a Kofler heating bench type 7841 apparatus and were uncorrected, ^{1} H and ^{13} C NMR spectra were recorded in $CDCl_3$ using a Bruker DRX-500 Mhz spectrometer. The mass spectrum was obtained with a Varian MAT CH-5 (EI) IR was obtained on a Perkin- Elmer SP3-257 spectrophotometer with polystyrene calibration at 1601cm^{-1}. UV spectra were determined on a Pye-Unicam SP8-400 UV/Vis spectrophotometer.

Extraction:

The method used by Rao et al (8) was employed with slight modification

The plant material 2Kg was macerated with methanol 95% in a blender and kept under the solvent for a period of 10 days. The extract was concentrated in a rotary evaporator at reduced pressure. The concentrate was extracted with toluene in order to remove chlorophyll. It was subsequently extracted with petroleum ether 60-80°C, benzene and ethylacetate (2liters each) in that order . The petroleum ether and the benzene fractions were subjected to further extraction using $CHCl_3$ (250 X 4)ml and did not yield any solid while the ethylacetate fraction gave a brownish mass on evaporation (2.5g), which showed the presence of four compounds on TLC. The mixture was chromatographed on a silica gel (100-200 mesh) 100g and eluted with gradient elution using benzene/petroleum ether. 20ml fractions were collected and fractions of the same R_f values were bulked together. Fractions 50-65 gave a crystalline compound melting point 178°C which was homogenous on TLC chloroform/methanol 9/1; R_f value of 0.78 and gave a positive reaction to chalcone.

Results and Discussion:

Phytochemical screening of the stem bark of Anthocleista djalonensis revealed the presence of Flavonoids, saponins, tanins and anthraquinones, while steroids and alkaloids and glycosides were in trace levels.(9, 10)

A crystalline amorphous compound AJEE1(400mg) wish was soluble in petroleum ether and benzene was isolated from the ethylacetate fraction.

Uv_{max} (MeOH) 382, 269, 230nm with $AlCl_3$ 386, 270, 229nm; with $AlCl_3$/HCL 390, 271, 213nm; with NaOAc 382, 269, 210nm

IR (KBr) cm^{-1} 3375, 1632, 1521, 1446, 1406, 1351, 1230, 1156, 1091

EI-MS m/z (relative intensity) 352(24) M^+, 337(42) M^+-CH_3, 309(27), 203(71), 187(100), 168(38), 131(45), 103 (25), 77 (34). High-resolution MS: m/z 352.1284; calculated for $C_{21}H_{20}O_5$: 352.1312.

The compound AJEE1 contained an enone moiety with an E-configurated C^a-C^b-double bond ($^3J_{HH}$=15.3Hz). A complete list of ^1H and ^{13}C chemical shifts are shown in table 1.

The long range ^{13}C, ^1H couplings (HMBC) prove the whole atomic network unequivocally. The phenyl ring attached to C^b carries a para-hydroxyl group and an additional methoxy group at C-3. The corresponding three proton -spin system fits to that structure in its typical ^1H chemical shifts and coupling constants. The substituents at the carbonyl group is a tetra-substituted benzoic ring with two ortho-positioned hydrogen (H-7' and H-8'). The isoprenyl residue contains a cis-configurated double bond as evidenced by the 10.0 Hz ^1H, ^1H coupling constant which is connected to the $(CH_3)_2C$-O group (d=77.8). It is attached to C-10' as proven by the observation of a long range correlation H-3'/C-10', where as the carbonyl group is fixed at C-6' (H-7'/C=O correlation). According to molecular formula, the isoprenyl oxygen is attached to C-9' forming a chromene ring. The C-5' hydroxyl ring is involved in a hydrogen bridge as shown by the ^1H chemical shift (d=13.8). Thus the structural part of AJEE1 attached to the C=O is identical to that which has been found very recently in the revised structure of crotaramosmin.(11)

A direct comparison of the spectra data of AJEE1 and the reference compound Polyarvin a chalcone from Polygala arvensis [1] showed them to be identical. (8)

The same structure have been reported as Pongachalkone-11 from the plant of Pongmia glabra, (12). Further evidence of its identification is based on a chalcone condensaation reaction followed by mixed melting point and co-TLC comparison as well as its mass spectrum.

[1]]

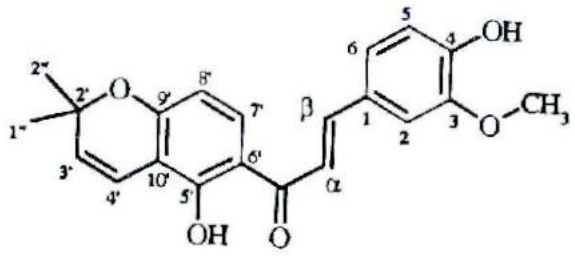

Table 1: ^1H and ^{13}C chemical shifts of AJEE1 including ^{13}C, ^1H long-range correlation's (HMBC; optimized to 7Hz); solvent: $CDCl_3$

	^1H	^{13}C	HMBC; ^{13}C-partners
1	-	127.4	-
2	7.12	110.1	3,4,6, b
3	-	146.8	-
4	-	148.4	-
5	6.96	114.9	1,3,47.23
6	7.23	123.5	2,4, b
A	7.40[a]	117.7	1, C=O
B	7.82[a]	144.6	1,2,6, a, C=O
C=O	-	191.9	-
2'	-	77.8	-
3'	5.59[b]	128.1	2',10',1"/2"
4'	6.76[b]	115.9	2',5',9'
5'	-	160.9	-
6'	-	114.1	-
7'	7.72	130.5	5',9', C=O
8'	6.38	108.2	6',9',10'
9'	-	159.7	-
10'	-	109.4	-
1"	1.47	28.4	2',3',2"
2"	1.47	28.4	2',3',1"
5'-OH[c]	13.8	-	-
OCH_3	3.97	56.0	3

[a] Vicinal ^1H, ^1H coupling constant J = 15.3Hz

[b] Vicinal ^1H, ^1H coupling constant J = 10.0Hz

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