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CHEMICAL CONSTITUENTS OF THE STEM BARK OF *ACACIA AURICULIFORMIS* A.CUNN EX. BENTH. (FABACEAE)

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ABSTRACT
The genus Acacia has been known to be a rich source of many secondary metabolites. This study was carried to isolate chemical constituents present in the stem bark of *Acacia auriculiformis*. The dichloromethane extract of the stem bark of *Acacia auriculiformis* was obtained by maceration. The extract obtained was subjected to silica gel column chromatography and preparative TLC. The isolated compounds were identified by spectroscopic analysis. This led to the isolation of ferulic acid ester (I), along with a steroid (II) and a triterpenoid (III). The structure of compound I was established using spectroscopic analysis (UV, IR, NMR and mass spectrometry) and identified to be dodecyl-4-hydroxy-3-methoxy-*trans*-cinnamate (I), compounds II and III were found to be á-spinasterol and lupenol respectively, based on the comparison of their spectral data NMR and MS with literature report. Compound I is being reported for the first time in the genus *Acacia*.

INTRODUCTION

Acacia is a large genus of the family Fabaceae, with approximately 1,400 species. Most of the species belonging to the genus are rich in secondary metabolites containing mainly tannins, flavonoids and gums [1], and are widely distributed in tropical and non-tropical countries including Nigeria. *Acacia auriculiformis* commonly referred to as Black Wattle, is an important medicinal plant and widely distributed member of the family Fabaceae. The Ibibio of Niger Delta region in Nigeria uses this plant as antimalarial remedy [2], an infusion of the bark of this plant is used to treat inflammation among the aborigines of Australia [3]. The antimutagenic and chemoprotective activities of *Acacia auriculiformis* as well as the ability of the ethyl acetate and acetone fractions to scavenge free radicals have been reported [4-6]. A new triterpenoid trisaccharide and three new triterpenoids have been isolated from this plant [7, 8]. The antimicrobial activity of acaciaside a and b the two saponins isolated from this plant was reported by [9]. The mosquito larvicidal activity of the fruit extracts of *Acacia auriculiformis* was recently reported [10]. As part of our investigation of the genus *Acacia* for protein kinase inhibitory agents we report herein the isolation from the dichloromethane extract of the stem bark of *Acacia auriculiformis,* a new ferulic acid ester, dodecyl-4-hydroxy-3-methoxy-*trans*-cinnamate named auriculate (I) along with α-spinasterol (II) and lupenol (III). The structure of compound I was elucidated using NMR and HR-ESI-MS, while compounds II and III were deduced based on the comparison of their spectral data with literature. Compound I is being reported for the first time in the genus *Acacia.*

RESULT AND DISCUSSION

Spectroscopic Analysis and Data

Compound I, a white solid (5.2 mg).

UV ($CHCl_3$): λmax(nm): 242 and 321

IR (KBr):Cm^{-1}: 3406 (OH) 2914.8-2847.7 (CH_2),1703.4 (conjugated C=O in ester),1155 (C-O in ester),1632 (C=C in alkene),and 1595.3,1613.3 (C=C aromatic ring). ^{1}H-NMR and ^{13}C-NMR (Table 1) HR-ESI-MS: *m/z* 361.9982 (M^+)

Compound I was isolated as a white solid, high resolution ESI-MS gave a molecular ion peak at *m/z* 361.9982 (m/z 362) which points to a molecular formula $C_{22}H_{33}O_4$ indicating a six degrees of unsaturation [11]. The UV spectrum gave λ_{max} at 242 and 321nm, respectively, which is typical of ferulic acid ester. The ^{1}H-NMR spectrum revealed a typical ABX spin system at δ=6.93 (d, *J*=8.0 Hz), 7.08 (d, *J*=2.0, 8.0Hz) and 7.05 (*d, J*=2.0 Hz) indicating the presence of three aromatic protons with ortho, ortho/meta and meta coupling respectively. The presence of a *trans* double bond was confirmed by signals at δ =6.29, d (*J*=17.0Hz) and 7.61, d, (*J*=17.0Hz) which was further confirmed by the H-H COSY which indicated correlation between the two protons. An oxymethylene and methoxy protons were evident at δ=4.19, t, *J*=6.7Hz) and 3.95, (s) revealed the compound to be a ferulic acid ester [12]. The deshielded nature of the oxymethylene signals confirms its linkage to the carbonyl moiety of the ferulate, while the 20H broad signal at δ at 1.30-1.32 ppm and the three proton triplets at δ 0.9 ppm were consistent with the presence of an *n*-dodecanyl long chain moiety, suggesting compound I to be a long chain ferulic acid ester. These spectral data suggestive of ferulic acid moiety was further supported by the ^{13}C-NMR spectrum. The ester carbonyl was evident at δ 167.4 ppm, and the side chain C-C double bond due to signals at δ 144.7 (C-7) and 115.7 (C-8).The oxymethylene, methoxyl and methyl carbons were evident from the signals at δ 64.6, 55.9 and 14.1 ppm respectively. Further confirmation of these carbons was resolved from the 2D experiments. The HMBC experiment

aided in the diagnostic linkages through the C-H long correlations. The vinylic proton at δ 7.61 (H-7) showed correlations with the carbon at δ 109.3 ppm assigned to C-2, 123.0 ppm assigned to C-6 and the carbonyl carbon at δ 167.4 (C-9), and also correlation between the vinylic proton H-8 with carbon signals at 127.1(C-1) and carbonyl signal at C-9 confirming the positions of the two vinylic carbons next to the carbonyl carbon (C-9). Correlation between H-6 proton at δ 7.08 ppm with carbon at δ109.3 ppm (C-2) further confirmed the presence of the ferulic acid moiety. The correlation between the oxymethylene protons with the carbonyl carbon and the methoxy protons correlating with signal at δ 146.9 (C-4) confirm the methoxy position to be at C-3 of the aromatic ring. Table 1 shows the complete C-H correlations. The mass spectrum of compound I which gave a molecular ion at *m/z* 362, revealed compound I to be a derivative of *n*-tetradecanyl ferulate reported by [13-14], and was named n-dodecanyl-3-methoxy-4-hydroxy-*trans*-cinnamate (auriculate). Compound II was isolated as a white solid. The mass spectrum gave a molecular ion peak of m/z 412 which points a molecular formula $C_{29}H_{48}O$ characteristic of a spinasterol moiety. The NMR spectra (^{1}H and ^{13}C) are in agreement with literature report of α-spinasterol [15, 16], while compound III was found to be lupenol by comparison of the NMR spectra with literature [17, 18].

Table1, ^{1}H and ^{13}C Spectra of compound I in $CDCl_3$

Carbon number	Chemical shift δ in ppm	δc in ppm	Some HMBC correlation
1	-	127.1	
2	7.05,1H,d (*J=2.0Hz*)	109.3	H-2 → C-4,C-6
3	-	147.9	
4	-	146.9	
5	6.93,1H,d,(*J=8.0Hz*)	114.7	H-5 → C-1,C-4
6	7.08,1H,d,d (*J=2,8Hz*)	123.0	H-6 → C-2,C-4
7	7.61.1H,d,(*J=16Hz*)	144.7	H-7 → C-1,C-9
8	6.29,1H,d,(*J=16*Hz)	115.7	H-8 → C-2 ,C-9
9	-	167.4	
OCH_2	4.19,2H,t (*J=6.7Hz*)	64.6	H-1^1 → C-9, C-2^1
OCH_3	3.95,s	55.9	OCH_3 → C-4
CH_3	0.9,3H,t(*J=6.8Hz*)	14.1	
C-11^1	1.71 (m)	22.7	
C-$3^{'}$	1.30,s,20H	26.0	
C-4’	1.30,s,20H	29.4	
C-5’ to C-8’	1.30,s,20H	29.7	
C-9’	1.30,s,20H	29.4	
C-10’	1.30,s,20H	31.9	

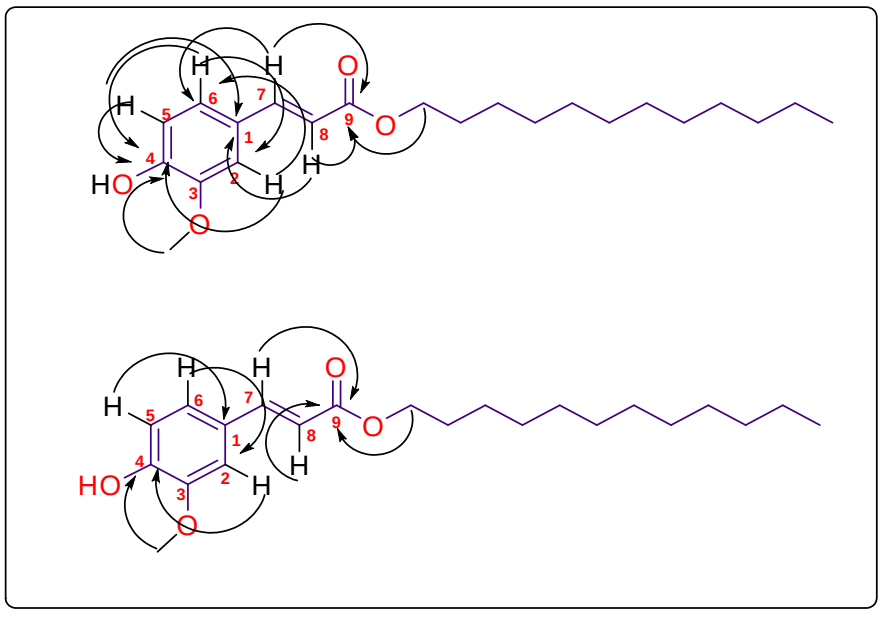


Figure 1: HMBC Correlations of Compound I

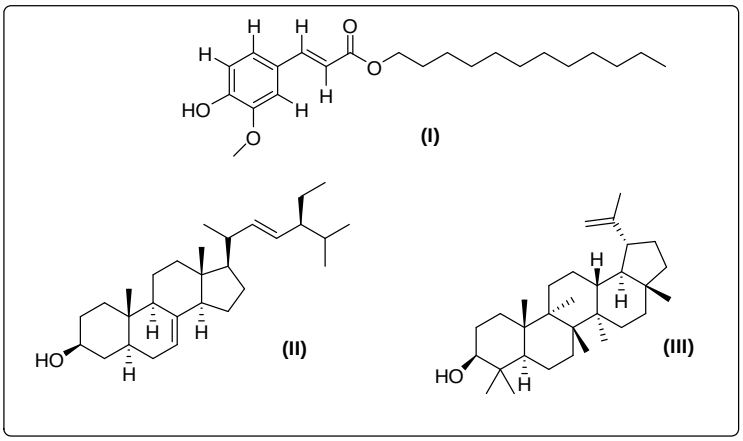


Figure 1: Chemical structures for Compounds I-III

CONCLUSION

Three compounds were isolated and identified from the stem bark of *Acacia auriculiformis*, which include a new natural ferulic acid ester derivative; *n*-dodecanyl-3-methoxy-4-hydroxy-*trans*-cinnamate alongside two other compounds, α-spinasterol and lupenol. These results further enriched scientific knowledge on the phytoconstituents present in *Acacia auriculiformis.*

MATERIALS AND METHODS

General procedures

Column chromatography was carried out on silica gel G (200-400mesh, Silicycle).Thin layer chromatography was performed on pre-coated TLC plates aluminium backed (0.2mm, Silicycle). NMR (^{1}H and ^{13}C) were performed on a Bruker Avance $400MH_Z$ (for ^{1}H) and $100MH_Z$ (for ^{13}C), while ESI-MS was performed on an ESI-LTQ orbitrap XL mass spectrometer (ThermoScientific, Germany).

Plant material

The plant material was collected in Zaria, Nigeria in July, 2016. The leaves and the bark where authenticated by B.D Musa of the herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen (number 1292) was deposited in the herbarium. The dried powdered bark (400 g) was extracted exhaustively with dichloromethane (1x2.5 L) and the removal of the solvent using a rotatory evaporator afforded a brownish sticky mass (2.2 g). The dried marc was exhaustively extracted with 2x2.5L of 70% ethanol at room temperature and removal of the solvent at reduced pressure afforded a brown solid mass (23.4 g).

Isolation

The dichloromethane extract (1.2 g) of the bark of *Acacia auriculiformis* was packed in a column (50 cmx3 cm) with 50 g of silica gel and eluted gradiently with *n*-hexane (100%), then n-hexane: ethyl acetate mixtures as follow: 99:1, 98:2, 97:3, 96:4, 95:5, 90:10, 80:20, 70:30, 60:40, 30:70, 10:90, ethyl acetate (100%) and finally 5% methanol in ethyl acetate.

The progress of elution was monitored on TLC using the solvent systems: *n*-hexane: ethyl acetate 15:1, 9:1, 5:1 and 3:1). Fraction 4, 50 mL eluted with 3% ethylacetate in *n*-hexane give a fatty material which was not further investigated. Fractions 10-13 eluted with 5% ethyl acetate in *n*-hexane afforded an orange solid (16 mg), while fractions 20-23 also eluted with 5% ethyl acetate in *n*-hexane afforded compound II (3.5 mg). Fractions 24-29 eluted with 10% ethyl acetate in *n*-hexane afforded compound I, a white solid (5.2 mg), while fractions 36-40 also eluted with 10% ethyl acetate in *n*-hexane were pooled together base on their similarity on TLC to give compound III, a pale white solid (12 mg).

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