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Research Article

Ferulic Acid Ester from The Stem Bark of *Acacia ataxacantha*

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ABSTRACT

From the chloroform extract of the stem bark of *Acacia ataxacantha*, a ferrulic acid derivative identified as n-tetradecanyl-3-methoxy-4-hydroxy –trans-cinnamate (tetradecanyl ferulate) compound 2 was isolated for the first time from this plant alongside the triterpene lupenol. The structure of compound 2 was confirmed using NMR and MS and is reported here for the first time. Primary activity of the isolated compound against a panel of disease related protein kinases reveal the compound to be in-active.

Keywords: *Acacia ataxacantha, tetradecanyl ferrulate, protein kinase*

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INTRODUCTION

Natural products and or natural product structures have continued to play a significant role in drug discovery and development process (Newmann and Cragg, 2016). Plants have continued to be a source of medicine especially in the developing parts of the world where about 80% of the population depend on natural products for their health needs (Rates, 2001; Calixto, 2000; Raskin *et al*., 2002). This has necessitated intensive studies into bioactive secondary metabolites using methods such as extraction, isolation, purification and chemical characterization of plant extracts which could unfold a novel chemical compounds suitable for drug development (Butler *et al*, 2004; Lahlou *et al*, 2007 Ebada *et al*, 2008).The therapeutic efficacy of medicinal plants in combating diseases lies in presence of Phytoconstituents present in them. These include alkaloids, flavonoids, steroids and saponins among others (Harbone, 1998; Busse, 2000; Newmann *et al*, 2000). Plants used as medicines have been considered safe, less toxic and also as a source of fantastic biomolecules unequalled by any synthetic procedure (Katiyar *et al*, 2012).

Human protein kinases represent the third largest enzyme class and are responsible for modifying an estimated one-third of human proteome (Manning *et al*,2002).It has been firmly demonstrated that hyperactivation, hyperproduction or mutations of these kinases leading to the disruption of cell signalling cascades, play important role in several diseases such as cancer, inflammation, diabetes among others, thus making protein kinases one of the most important targets for pharmaceutical industry (Capedeville *et al*, 2002). The human genome consists of over 517 protein kinases that include two sub families, serine/threonine and tyrosine kinases (Capedeville *et al*, 2002). Numerous tyrosine kinases inhibitors have been discovered by screening plant extracts based on ethnopharmacological and chemotaxonomic knowledge (Hollosyl and Keri, 2004). Specific screening approach have led to the isolation of structurally distinct classes of inhibitors which have served as leads for further design and synthesis of more active analogues.

Acacia is a large genus of the family fabaceae, with about 1,400 species. Most of the species belonging to the genus are rich in secondary metabolites containing mainly tannins, flavonoids and gums (Seigler, 2003), and is widely distributed in tropical and non-tropical countries including Nigeria. Acacia ataxacantha is wide spread in sub Saharan Africa including Nigeria, Benin and Kenya where various parts of the plant has ethnomedicnal applications in relieving ailments such as dysentery, cough, joint pains and pneumonia(Mac Donald *et al*,2010;Kareji,2013). The antioxidant, antifungal and antibacterial activity of the bark extracts have been reported (Amoussa *et al*, 2015 a,b). Phytochemically, triterpenoids and a new chromone have been reported by (Amoussa *et al*, 2016, a b). As part of our continuing studies of the genus Acacia, for protein kinase inhibitory constituents, we report herein the isolation of a ferrulic acid ester from the

chloroform extract of the stem bark of Acacia ataxacantha and the primary screening of the compound against a panel of disease related protein kinases.

MATERIALS AND METHODS

Column chromatography was performed on silica gel G 200-400 mesh (Silicycle),Thin layer chromatography was performed on pre-coated TLC plate silica gel (0.2 mm) aluminum backed (Silicycle). UV spectrum was performed on Pye-Unicam UV Spectrophotometer. IR spectra were recorded on an Agilent 2346, Infra-Red spectrophotometer in Kbr. NMR spectra (1H and 13C) were performed on a Bruker DRX 400 spectrophotometer (400MHz for 1H and 125MHz for 13C) in $CDCl_3$ using TMS as internal solvent and high resolution mass spectrometry was carried out using an ESI-LTQ-orbitrap Discovery XL mass spectrometry (Thermo Scientific, Germany).

Plant material

The stem bark of Acacia ataxacantha was collected in Zaria in the month of August and authenticated at the herbarium section, Biological Science Department, Ahmadu Bello University, Zaria-Nigeria, was a voucher specimen (900290) was deposited in the herbarium.

Extraction and Isolation

The powdered bark of the plant (250 g) was extracted at room temperature to exhaustion using maceration with 2.5L of chloroform; the combined chloroform extract was concentrated to give a brownish mass (2.3 g). The marc was then extracted with 2x 2.5 L of 70% ethanol and the combined ethanol extract after the removal of the solvent using rotary evaporator gave 17.2 g of ethanol extract. 1.8 g of the chloroform extract of the bark of Acacia ataxacantha was packed in a column (1.9cm x 42cm) and eluted gradiently with n-hexane and n-hexane ethylacetate mixtures. The progress of separation were monitored on TLC using the solvent systems N-hexane:ethylacetate (9:1; 5:1 and 3:1). Fractions eluted with 5% ethylacetate in n-hexane afforded from 4 pooled fractions (25 ml) and removal of the solvent afforded compound 1, a white solid (7 mg). Fractions eluted with 20% ethylacetate in n-hexane afforded from the pooled fractions (20 ml) upon removal of the solvent a pale yellow solid which was re-purified over sephadex LH-20 eluted with 5% n-hexane in dichloromethane to give compound 2, a white solid (9 mg).

Protein Kinase inhibitory studies

Compound 2 was screened against a panel of disease related protein kinases. Kinase activity were assayed in appropriate buffer, with either protein or peptide as substrate in the presence of 15 µM [ɣ-33P]ATP (3,000 Ci/mmol, 10mCi/ml) in a final volume of 30 µl following the assay described by (Bach *et al*, 2005). Controls were performed with appropriate dilutions of dimethyl sulphoxide. Full length kinases were used unless specified.

RESULTS

Compound 1 was found to be Lupenol by comparison of its spectral properties (1H and 13C-NMR and MS) with literature data (Privitea and Monaco, 1984; Connolly and Hill, 1999) and as previously reported from this plant (Ahmadu *et al, 2018*).

Compound 2, a white solid (9 mg). UV ($CHCl_3$): λmax. 235, 318nm

IR(KBr):cm^{-1}: 3444; 2852; 1737; 1682; 1639; 1599; 1451-1376; 1073; 1043 and 722.

^{1}H-NMR ($CDCl_3$): δ (ppm),7.63 (1H, d,J=17Hz),7.10 (1H, d,d,J=2,8.5Hz), 7.05 (1H,d,J= 2Hz, 6.85 (1H,d,J=8.5Hz), 5.85 (1H, s), 5.2 (1H,s),4.21 (2H, t, OCH_2), 3.90 (3H,s,OCH_3), 1.55 (m),1.25 (10H, s), 0.90 (3H, t, J = 6.8Hz)

^{13}C-NMR ($CDCl_3$): δ (ppm): 179.5, 167.5, 147.9, 144.7, 127.0, 123.1, 115.6, 114.7, 109.2, 64.5, 55.9, 34.0, 32.0, 29.7, 29.4, 27.2, 26.0, 24.7, 22.7 and 14.2

HR-EI-MS: m/z 392.2322 (M^++2)), 391.2298(M^++H), 377.2144, 194.1112, 177.2013

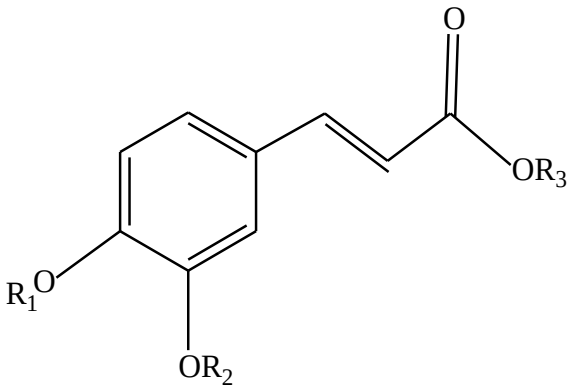

Figure 1
Compound 2,R_1=H;R_2=Me;R3=-CH_2 $(CH_2)_{12}CH_3$

Table 1 shows the results of the primary screening performed using 50 µg/ml of compound 1. Data are expressed in percentage of maximal activity measured in the absence of the inhibitor. ATP concentration used in the kinase activity was 15 µM (values are mean: n=2). Kinases are from human (Hs-Homosapiens; Ssc: Sus Scrofa; Rn: Ratus norvegicus; Mm: Mus musculus; Pf: Plasmodium falciparum; Lm: Leishmania major; Ld: Leishmania donovani.

Table 1
Primary activity of compound 2 against disease related Protein kinase#

Compound	HsCdk2	HsCdk5	HsCdk9	MmCLK1	SscGSK3	RnDYyrk1A	PIM1	Haspine	SscCK1	LmCK1	Hs AURK B
1	76.0	70.0	68.0	48.0	66.0	63.0	84.0	108.0	45.0	71.0	76.0

DISCUSSION

Compound 1, was isolated as a white solid, its spectral properties (NMR and MS) are in agreement with the literature data of Lupenol (Chaturvedula and Indira, 2012).

Compound 2, was isolated as a pale white solid. High resolution ESI-MS gave an (M+1) peak at 391.2298 which points to a molecular formula $C_{24}H_{38}O_4$, indicating six degrees of unsaturation. The diagnostic fragment peaks at m/z 177 and 194 are both characteristic of a methoxy and hydroxyl-substituted cinnamic moiety (Achenback *et al*, 1986). The presence of double bond was evident from the IR bands at 1639,1599cm-1, while a carbonyl band of an ester appeared at 1737cm-1.

The UV spectrum gave λmax at 235 and 318nm typical of ferrulic acid ester (Delazar *et al*, 2004)). The proton NMR spectrum in CDCl3 revealed a typical ABX spin at system at δ 6.85(d, J=8.5Hz), 7.10 (d, d ,J=2, 8.5Hz) and 7.05(d,J=2Hz) 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 6.27 d (J=17Hz and 7.63 d (J=17Hz) respectively, while an oxymethylene and methoxy protons at δH 4.21 (t) and 3.90(s) revealed the compound to be a ferulic acid ester, a tetradecanoyl moiety was evident with the signals at (δ=0.9,t, J=6.8Hz;1.25,s, (CH_2X_8), 4.21,t, J=6.8 Hz). The deshielded nature of the oxymethylene signal at δ=4.21 (t) confirmed its link to the carbonyl of the ferulic acid moiety and thus provided evidence for the ester formation (Delazar *et al*, 2004), a long chain methylene signal at δ=1.25 (s) was also evident from the ^{1}H-NMR spectrum. The ^{13}C-NMR spectral data further confirmed the presence of ferulic ester moiety with ester carbonyl at δ=167.7 ppm, methoxyl signal at δ=55.9 ppm, oxymethylene carbon at δ=64.5 and methyl carbon at δ=14.1 ppm respectively.

The ^{13}C-NMR spectral data assignment was further resolved through the DEPT 135 experiment into four quaternary carbons at δ=167.5,147.9,146.8 and 127.0 ppm assigned to (C-9, C-4,C-3 and C-1) of the ferulic acid moeity, 5 methine carbons at δ=144.7,123.08,115.62,114.64,109.23, were assigned to (C-7,C-6,C-2,C-5 and C-8),while the methyl and methoxy carbons were evident from the DEPT 135 spectral which depicted the signals at δ=55.9 and 14.2 ppm respectively. The oxymethylene carbon at δ=64.67 ppm was also confirmed through the DEPT experiments, while the long chain methylene carbon signal was confirmed at δ=29.7 ppm. The ferullic acid moiety of compound 2 compared very well with a similar ferulic acid ester reported by (Delazar *et al*, 2004). All the spectral data of compound 2 compares very well with the only report in the literature of this compound reported by (Nkengfack *et al*,1997) from Erythina species. This is the first report of this long chain ferulic acid ester from the genus Acacia. Compound 2 was screened for primary activity against a panel of disease related protein kinases (Table 1), the result revealed the activity that remains in the tube after treating the kinases with 50 µg/ml of compound 2 compared to the control assay that was treated with DMSO. The result indicated that compound 2 was not active against most of the kinases for example against PIM 1; it shows only 16% of the kinase activity, while the highest activity was against CK1 which gave 55% inhibition of the enzyme activity. This result revealed that compound 2 is inactive against the protein kinases investigated because most of the activity of the compound which gave less than 50% of the kinase activity remaining in the tube is only two, against CLK1 and CK1 out of the eleven protein kinases investigated.

In conclusion, ferulic acid ester was isolated for the first time from this plant and protein kinase inhibitory study revealed it to be in-active against the disease related kinases investigated

Conflict of Interest Disclosure:
The authors declare that they have no competing interest

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