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Chemical constituents of the Stem Bark of *Acacia ataxacantha* (Fabaceae)

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ABSTRACT

Medicinal plants have served as source of lead discovery in drug development. Human protein kinases represent the third largest enzyme class and are responsible for modifying an estimated one-third of human proteome. Numerous tyrosine kinases inhibitors have been discovered by screening plant extracts based on ethnopharmacological and chemotaxonomic knowledge. 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.
The purpose of this work is to screen phytochemically the constituents of *Acacia ataxacantha* as part of our continuous study on the Phytoconstituents of the genus Acacia for protein kinase inhibitory agents. The chloroform extract of the stem bark was subjected to silica gel column chromatography and the eluates were monitored using thin layer chromatography leading to the isolation of compounds 1 and 2. Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) were used to elucidate the structures of the isolated compounds. Compound 1 was isolated as a pale white solid, and its spectral data was consistent with that of lup-20(29)-en-3β-ol *(lupenol)*, while compound 2 was found to be stigmasta-7, 22-dien-3*β*-ol (*α-spinasterol*) by comparing its spectral data with literature. This is the first report of these compounds from *Acacia ataxacantha*.

Keywords: *Acacia ataxacantha*, lupenol, α-spinasterol, protein kinase.

Introduction

Natural products and or natural product structures have continued to play a significant role in drug discovery and development process.[1] 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.[2-4] 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.[5-7] 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,[8] and is widely distributed in tropical and non-tropical countries including Nigeria, Benin and Kenya where various parts of the plant has ethnomedicinal applications in relieving dysentery, cough, pneumonia,pain and inflammation.[9,10] The antioxidant, antifungal and antibacterial activity of the bark extracts have been reported.[11,12] Phytochemically, triterpenoids and a new chromone have been reported.[13,14] We have previously reported the protein kinase inhibitory activity of two new Peltogynoids isolated from the chloroform extract of the stem bark of *Acacia nilotica*,[15] while the ethylacetate soluble fraction of the stem bark of *Acacia nilotica* afforded a novel protein kinase inhibitory activity of two phenolic compounds isolated.[16] As part of our continuing studies of the genus Acacia, for protein kinase inhibitory constituents, we report herein the isolation of the triterpenoid: lupenol and α-spinasterol from the chloroform extract of the stem bark of *Acacia ataxacantha*, the two compounds were inactive against a panel of disease related protein kinases.

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Materials and Methods

General experimental procedures
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). NMR spectra (^{1}H and ^{13}C) were performed on a Bruker DRX 400 spectrophotometer (400MHz for proton and 125MHz for 13C) in $CDCl_3$ using TMS as internal standard, while ESI-MS 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 2017 and authenticated at the herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where 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 for seven days by maceration with 2.5 L of

chloroform; the combined chloroform extracts was concentrated to give a brownish mass (2.3 g). The marc was then extracted with 2x2.5L of 70% ethanol and the combined ethanol extract after the removal of the solvent using rotary evaporator gave 17.2 g of brownish mass of ethanol extract.

A portion of the chloroform extract (0.5 g) was packed in a column (1.9 x 42 cm) and eluted gradiently with n-hexane and n-hexane ethylacetate mixtures as follow: n-hexane (100%), 95:5, 90:10, 80:20, 70:30, 50:50, 30:70, 10:90 and ethaylacetate (100%). The progress of separation was monitored on TLC using the solvent systems N-hexane: ethylacetate (9:1 and 5:1). Fractions eluted with 10% ethyl acetate in n-hexane afforded compound 1, a pale white solid (6 mg), while fractions eluted with 20% ethylacetate in n-hexane afforded compound 2 a white solid (4.5 mg).

Protein kinase inhibitory studies

Compounds 1 and 2 were screened against a panel of diseased related protein kinases. Kinase activity were assayed in appropriate buffer, with either protein or peptide as substrate in the presence of 15μM [γ-33P] ATP (3,000 Ci/mmol; 10 mCi/mL) in a final volume of 30 μL following the assay described by Bach *et al*., 2005.[17]

Controls were performed with appropriate dilutions of dimethyl sulphoxide. Full length kinases were used unless specified. Peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

Results and Discussion

Compound 1 was isolated as a pale white solid (6 mg). LC-MS: m/z: 426.31 (18%, M^+), 411.32(10%, M^+-CH_3), 207.08 (100%), 189.15 (78%). ^{1}H-NMR ($CDCl_3$,400MHz), δ(ppm): 0.66 (1H, d, *J* = 8.5 Hz, H-5), 0.74 (3H, s, Me-28), 0.81 (3H, s, Me-25),0.92 (3H, s, Me-27), 0.94 (3H, s, Me-23), 1.01 (3H, s, Me-20), 1.66 (3H, s, Me-30), 2.36 (1H, m, H-19),3.17 (1H, d, d.J=10.2,5.1Hz,H-3), 4.55 (1H, br s, H-29a), 4.67 (1H, br s, H-29b). ^{13}C-NMR ($CDCl_3$, 100 MHz), δ(ppm): 38.7 (C-1), 27.4(C-2), 79.0 (C-3), 38.9 (C-4), 55.3 (C-5), 18.4 (C-6),34.3 (C-7), 40.0 (C-8), 50.9 (C-9), 37.2 (C-10), 21.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 46.3 (C-18), 48.0 (C-19), 151.0 (C-20), 30.0 (C-21), 40.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.5 (C-27), 18.0 (C-28), 109.3 (C-29), 19.3 (C-30).

Compound 2, a white solid (4.5 mg). It gave a retention time of 40.52 min from the total ion chromatography. LC-MS, m/z: 412.28 (20%, M^+), 397.27 (15%, M^+-CH_3), 271.16 (100%), 255.19 (40%), 81 (85%) ^{1}H-NMR ($CDCl_3$): δ (ppm): 0.58 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 0.81 (3H, t, *J* = 7.0 Hz 27-CH_3), 0.86 (3H, d, *J* = 6.4 Hz, 26-CH_3), 1.06 (3H, d, *J* = 6.5 Hz, 21-CH_3), 1.3 (1H, s,), 1.6 (2H, s), 3.67-3.73 (1H, m, 3-CH), 5.05 (1H, dd, *J* = 15.2, 8.6 Hz, 23-CH), 5.17 (1H, s, 7-CH), 5.18 (1H, d, *J* = 6.5 Hz, 22-CH_3). ^{13}C-NMR ($CDCl_3$) δ(ppm): 37.8 (C-1), 29.4 (C-2), 72.2 (C-3), 35.2 (C-4), 40.7 (C-5), 31.6 (C-6), 118.5 (C-7), 139.5 (C-8), 50.5 (C-9), 35.0 (C-10), 22.1 (C-11), 40.1 (C-12), 44.0 (C-13), 55.8 (C-14), 22.3 (C-15), 26.2 (C-16), 56.6 (C-17), 12.0 (C-18), 13.5 (C-19), 41.5 (C-20), 21.9 (C-21), 141.5 (C-22), 129.0 (C-23), 51.9 (C-24), 32.7(C-25), 19.7 (C-26), 21.5 (C-27), 24.6 (C-28), 13.0 (C-29).

Compound 1, was isolated as a pale white solid, mass spectral data revealed a molecular ion peak at m/z 426.31 which is consistent with the molecular formula $C_{30}H_{50}O$ suggestive of a triterpene, and the fragment peak at m/z 189 is typical of a lupine type triterpene.[18] The ^{1}H-NMR spectrum displayed a characteristic signal of a downfield singlet at δ1.66 ascribable to the vinyl methyl proton at (C-30), and a pair of broad singlets due to exomethylene protons (H-29) at δ = 4.55 and 4.67 ppm consistent with isopropenyl side chain of lupine type triterpenoid. [19]The doublet of doublets signals at δ=3.17 in the spectrum was typical of a 3-hydroxy type triterpenoid. The proton NMR also showed seven methyl singlets at δ=0.74, 0.76, 0.81, 0.92, 0.94, 1.01 and 1.66 and a secondary hydroxyl methine at δ = 3.17. The ^{13}C-NMR spectrum showed 30 signals for triterpenoid of lupine skeleton with the hydroxyl methine carbon signal evident at δ=79.0, while the olefin carbons of the exocyclic double bond appeared at δ151.0 and 109.3 respectively. The (NMR and MS) are in agreement with the literature data of Lupenol.[20]

Compound 2, was isolated as a white solid. The ESI-MS gave an (M^+) peak at 412.28 which is consistent with the molecular formula $C_{29}H_{48}O$ suggestive of a steroidal nucleus, with fragmentation peaks at m/z 397, 300, 271, 255 and 81. Loss of 141 amu from the molecular ion peak correspond to the loss of lateral aliphatic chain of sterol (m/z 141) to give a fragment peak at m/z 271 which correspond to the base peak.[21] The ^{1}H-NMR spectrum of compound 2 revealed the presence of signals at δ=5.04, 5.17 and 5.18 ppm indicating the presence of double bonds. A multiplet signal at 3.67 ppm indicates the presence of hydroxyl methine proton. The ^{13}C-NMR spectrum further confirms the olefin carbon at δ = 118.4, 129.5, 139.5 and 141.5 ascribable to the double bond, while the signal at δ=72.2 ppm further confirm the presence of hydroxymethine carbon. HSQC experiment further revealed the C-H correlations of the signal at δ = 3.67 ppm correlate with 72.2 ppm, 5.05 correlate with 129.0 ppm, 5.17 with 118.5 ppm, and 5.18 with 141.5 ppm which corresponds to C-23, C-7 and C-22 of the sterol nucleus, while the signal at δ=139.5 ppm is a quaternary carbon. The H-H cosy experiment further provide correlation between proton at δ = 5.05 ppm with proton at 5.18 ppm confirming double bond with carbon signals at δ = 141.5 and 129.0ppm corresponding to C-22 and C-23 indicating that the protons are neighbor. Correlation was also observed between proton at δ=3.67 with proton at 1.6 ppm corresponding to the bond of C3 and C-4. Proton correlation at δ=2.05 with proton at 1.06 ppm indicate a direct correlation of C-17 and C-20 carbon between the steroid nucleus and aliphatic lateral chain[21]. The HMBC experiment revealed correlation between proton at δ = 0.58 (H-18) with carbon at δ=40.5 (C-12) and 55.1 (C-14/17), correlation between proton at 0.81 (H-19) with carbon at 37.5 (C-4) and 50.5 (C-9), while correlation was also observed between proton at 5.05 (H-23) and carbon signals at 42.0 (C-13), 55.1(C-14). Correlation was also observed between proton at 0.86 (H-26) with carbon signal at 51.9 (C-24), 32.7 (C-25) respectively. The HMBC experiment also revealed correlation between proton at 5.18 (H-22) with carbon signal at 24.6 (C-28), 55.8 (C-14). This allowed compound 2 to be unequivocally assigned as the structure of α-spinasterol, the NMR and MS spectral data is consistent with that of α-spinasterol in literature.[21, 22] Compounds 1 and 2 have been reported from other species of Acacia, [23] to the best of our knowledge, this is the first report of these compounds from *Acacia ataxacantha*.

Table 1 shows the activity that remains in the tube after treating the kinases with 50μg/ml of the two compounds compared to the control assay treated with DMSO. The results show that compound 1 was inactive against most of the kinases for example against CLK1 it shows only 1% of the kinase activity, while the highest activity was against GSK3 which gave 46% of GSK 3 kinase activity. Compound 2 in contrast gave the highest activity against TLK kinase with 78% of TLK activity, while against CLK1; it gave 11% of the kinase activity. This result shows that the two compounds are not active against the protein kinases investigated as most of the activity of the compounds which gave less than 50% of the kinase activity remaining in the tube is only two for compound 2, and none for compound 1.

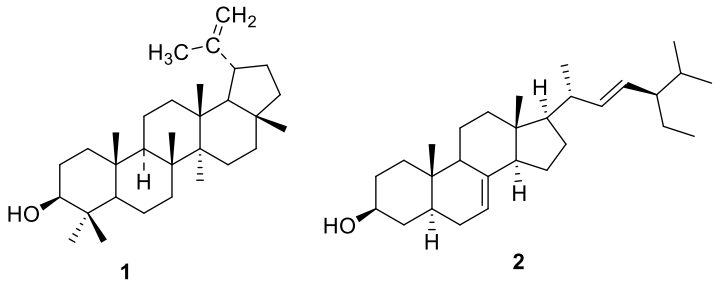

Figure 1: Structures of isolated compounds.

Table 1: Primary activity of isolated compounds against 11 disease related Protein kinase.

Compound	HsCdk 2	HsCdk 5	HsCdk 9	MmCLK 1	SscGSK 3	RnDYyrk1 A	PIM 1	Haspin e	LdTL K	LmCK 1	HsAurora B
1	110.0	106.0	77.0	99.0	54.0	85.0	102.0	115.0	142.0	74.0	117.0
2	109.0	104.0	53.0	89.0	73.0	74.0	96.0	122.0	22.0	87.0	97.0

The table reports the results of the primary screening performed using 50 µg/mL of the compounds. Data are expressed in % of maximal activity measured in the absence of the inhibitor. ATP concentration used in the kinase assay was 15 µM (values are means: n = 2). Kinases are from human (Hs - *Homo sapiens*; Ssc - *Sus Scrofa*; Rn - *Ratus norvegicus*; Mm - *Mus musculus*; Pf - *Plasmodium falciparum*; Lm - *Leishmania major*; Ld - *Leishmania donovani*.

Conclusion

Two compounds were isolated for the first time from the chloroform extract of the stem bark of *Acacia ataxacantha*. The two compounds were found to be inactive against a panel of disease related protein kinases, suggesting that the activity might be in the polar extract which is currently being investigated.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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