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Isolation and characterisation of cupressuflavone from the leaves of *Lophira lanceolata*

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Abstract
The leaf of *Lophira lanceolata* (Ochnaceae) is used by traditional medicine practitioners in Africa and other parts of the world to treat dysentery. Preliminary phytochemical examination of the extracts of the leaves of *Lophira lanceolata* indicated the presence of flavonoids, anthraquinones, cardiac glycosides, tannins, saponins and free reducing sugar while alkaloids are absent. The acetone extract of the plant's leaves was subjected to extensive column and thin-layer chromatography on silica. This afforded a compound characterized as cupressuflavone, a biflavonoid. The structure was elucidated using spectroscopic techniques, - UV, IR, NMR (2-D) and MS (Electrosspray Ionisation – ESI). Spectroscopic data obtained are consistent with literature values for cupressuflavone. Preparation of methylated and acetylated derivatives was carried out, for confirmation.

Keywords: *Lophira lanceolata*; Ochnaceae; Cupressuflavone; Biflavonoid

Introduction

The plant *Lophira lanceolata* (family Ochnaceae) is a spermatophyte used in treating dysentery and other intestinal problems. The following compounds have been reported to be found in the leaves of *Lophira lanceolata.* They include: flavanol, isoflavone and flavanonol (Tih *et al.*, 1994); lophiraic acid and its derivatives; lophirone A and its derivatives (Akira *et al.*, 1991)

Although there are several examples of naturally occurring biflavonoids and biisoflavonoids, the dimeric compounds consisting of a flavone or isoflavone and a heterocyclic compound like an indole, benzofuran or chromene are not known in the literature. Furthermore, heterocyclic dimers containing one flavone and one isoflavone molecules have not been reported in the literature.

The aim of the present work therefore, was to isolate and characterize some constituent compounds from the acetone extract of the leaves of *Lophira lanceolata.*

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Experimental

Plant material. The leaves of *Lophira lanceolata* was collected from, Sakaru village along Jos road Zaria, in December 2005 and identified at the Herbarium section, Biological Science Department, Ahmadu Bello University Zaria as *Lophira lanceolata* a voucher coded 4002B was deposited in the Herbarium. [Species: *Lophira lanceolata* Van Tiegh. Ex Keay (Letouzey 1968, 1985)**].**

Extraction and isolation. The dried, ground powder (1 kg) was extracted to exhaustion using acetone by cold maceration (Ohta and Yagishita, 1970). The combined acetone extract was concentrated to give a dark brownish mass, labeled extract 'A'.

The extract was separated by a combination of chromatographic techniques. Thin-layer chromatography (TLC) was conducted on pre-coated, commercial silica gel (Merck, 60 GF_{254}) plates with several developing solvent systems. Accelerated Gradient Chromatography, a form of Medium Pressure Liquid Chromatography (MPLC) was carried out on silica gel (Merck, Kieselgel 60, 60-230 mesh). Open column gel filtration chromatography over Sephadex LH-20 (Pharmacia) was also employed. These techniques were combined to give a compound coded "ALK1". Structure elucidation from spectroscopic data strongly suggested that the compound is cupressuflavone, a biflavonoid.

Spectroscopy. Ultraviolet (UV), infrared (IR), and Electrospray ionization (ESI) Mass Spectroscopy (MS) measurements were taken on Perkin Elmer instruments. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AMX 300.

Derivatives. A portion of 'ALK1' was methylated with potassium carbonate and dimethyl sulphate in dry acetone. The mixture was refluxed for 8 hrs. After work up, the product, coded 'AM1' crystallized from $CHCl_3$ as colourless needles. Another portion of 'ALK1' was acetylated with pyridine and acetic anhydride. The acetate, after usual work up crystallized from $CHCl_3$-MeOH as colourless needles which was coded 'AT1'.

Preliminary phytochemical screening. The presence of various classes of plant constituents were tested using a small portions of the extract. The tests were carried out in accordance with standard procedures ().

Results and Discussion

The physical characteristics and spectroscopic data of the isolated compound are detailed below.

Biflavonoids are primary coloured pigment and moderately polar compounds due to the presence of various hydroxyl groups on the nucleus. They also gave positive colour test for flavonoid. Cupressuflavone has four hydroxyl groups with R_f of 0.30 in Benzene-Pyridine-Formic acid (BPF).

Physical properties of ALK1 (cupressuflavone)

[I-4', II-4', I-5, II-5, I-7, II-7-Hexa hydroxy (I-8,II8) – Biflavone]

m.p = 168– 169^{o}C

Elemental analysis. Found -66.91%C, 3.3%H.
Calculated - 66.91%C, 3.3%H.

Molecular formula: $C_{30}H_{18}O_{10}$

UV λ_{max} MeOH (nm): 282, 326; +$AlCl_3$: 283, 355; +$AlCl_3$/HCl: 283, 347; +CH_3ONa: 235, 285, 391; +CH_3COONa : 282, 343, 383; + CH_3COONa/H_3BO_3 285, 333.

I.R (KBr) (cm^{-1}) 3444, 1648, 1589, 1546, 1508, 1192, 1145, 1070, 835 cm^{-1}

: 3444, (OH), broad band around: and 1648 (C=O).

3444 = OH; alpha/beta unsaturated C=O (1648); and aromatic (1589 and 1546) cm^{-1}

^{1}H NMR data of ALK1 in DMSO

Position	δ (ppm)
H-3	6.783s
H-6	6.444s
H-8	---------
H-2’	7.501d (8.8)
H-3’	6.75d (8.8)
H-5’	6.756d (8.8)
H-6’	7.501d (8.8)
H-3’’	6.783s
H-6’’	6.444s
H-8’’	-------
H-2’’’,6’’’	7.501d (8.8)
H-3’’’,5’’’	7.4726d (8.8)
OH-4’,4’’’	12.89s
OH-5 ,5’’’	13.16s
OH-7 ,7’’’	12.89s
7-OMe	

^{13}C NMR data of ALK1 in DMSO

δ (ppm)	Position
99.167	(C- 6)
99.254	(C-6’ , 6’’_)
103.023	(C-3’ ,3’’_)
104.056	(C-10 ,10’’),
116.274	(C-3’ ,5’ , 3’’’,5’’’)
121.682	(C-1’ , 1’’’_)
128.393	(C-2’ ,6’ 2’’’ ,6’’’)
155.233	(C-9,9’’_)
161.298	(C-7,7’’)
161.488	(C-5,5’’_)
163.966	(C-2,2’’_)
182.510	(C-4,4’’)

MS/ESI PEAKS(ALK1) Molecular ion peak 538 m/z 300MHz

257.05 , 279.11 , 309.17 , 331.15 , 334.04 , 373.05 , 375.12 , 376.13 , 377.18 , 387.16 , 399.11 , 417.11 , 418.14 , 419.07 , 443.07 , 444.09 , 451.07 , 469.00 , 493.04 , 519.17 , 537.06***** (Molecular ion) , 538.08 , 539.07 , 550.10 , 566.16 , 592.95 , 611.11

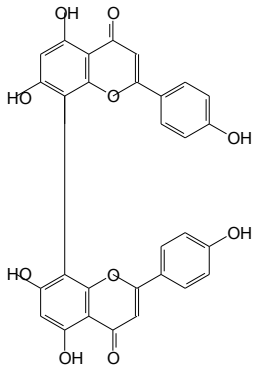
MS/ESI PEAKS (ALK1) M^{-1}

257.05 , 279.11 , 309.17 , 331.15 , 373.05 , 375.12 , 377.18 , 387.16 , 399.11 , 417.11 , 419.07 , 443.07 , 451.07 , 469.00 , 493.04 , 519.17 , 537.06****** (Molecular ion) , 539.07 , 611.11

MS/ESI PEAKS(ALK1) Molecular ion peak M^{+1}

257.19 , 311.12 , 344.90 , 367.18 , 399.15 , 420.94 , 443.02 , 459.29 , 460.31 , 507.31 , 531.46 , 551.30 ,575.38 , 595.38 , 619.43 , 639.41 , 663.48 , 683.48 , 685.48 , 686.66 , 727.53 , 729.48 , 730.51 , 765.57 , 771.54 , 773.50 , 775.52 , 776.54 , 779.55 , 815.55 , 817.55 , 818.55 , 819.59 , 823.40 , 859.57 , 861.61 ,863.53 , 873.53 , 903.61 , 905.57 , 907.59 , 908.61 , 917.58 , 947.64 ,949.61 , 951.65 , 952.63 , 969.63 , 991.61 , 993.60

Methyl derivative: m.p. 182
Acetate derivative: m.p. 183-184



Cupressuflavone

The absorption affinity differences index increases with increasing methylation and acetylation so much so that fully methylated biflavone involving various modes of interflavonyl linkage were found to show sizeable difference in R_f values. The different shades of the spots of these fully methylated derivatives in U.V. light was also found to be of some help in their identification.

The structure of cupressuflavone in *Lophira lanceolata* was determined by TLC, UV, IR, MS and NMR. The UV indicated various ranges within the limit of Biflavonoids. The (M^+) MS for the compounds did not give the mass of the compounds with respect to the UV observed hence the (M^-) ESI/ MS and IR were conducted which gave a clearer picture of the compounds. IR showed a band at 3444 cm^{-1}, for (OH), and a broad band around 1648 cm^{-1} for (C=O). From the mass spectra, seven compounds can be suggested to have the same mass and reactions, they are $C_{30}H_{18}O_{10}$, $C_{31}H_{22}O_9$, $C_{32}H_{26}O_8$, $C_{33}H_{30}O_7$, $C_{34}H_{34}O_6$, $C_{35}H_{38}O_5$, $C_{36}H_{42}O_4$. On spectral analysis of the C^{13} and ^1H NMR of ALK1, it indicated that the only possible compound is a biflavonoid with 8-8" linkage as there was no peak observed for carbon 8 in the C^{13} and proton at position 8 in the ^{1} H NMR. The isolation of this biflavone was carried out from the acetone extract by column chromatography using silica gel to give fraction 'C1' The fraction was purified by re-chromatography on Sephadex LH-20 column (MeOH).

Thus the structure was elucidated on the basis of TLC, UV, IR, ESI-MS and by comparison of ^{1}HNMR spectra with the literature data (Geiger and Markham, 1996; Krauze-Baranowska *et al.*, 1999; Markham, 1982; Wollenweber *et al.*, 1998). In the ^{13}C NMR spectrum of compound ALK1 two carbon signals with very similar values of chemical shifts at 99.8 and 99.6 ppm characteristic for non-substituted carbon in position C-6 of biflavone (Markham, 1982) were observed, that confirmed the presence of C8-C8" bond of biapigenin skeleton. Regarding the data of NMR spectrum the above signals have been distinguished as not substituted carbon C-6 (99.8 ppm) and substituted carbon in position C-8 (98.6 ppm). This observation is a new one against earlier reports (Markham, 1982; Wollenweber *et al.*, 1998) that demonstrated the downfield shift of the carbons C-8/C-8" of cupressuflavone below 100 ppm (between 102- 104 ppm).

Conclusion

Cupressuflavone, a biflavonoid, was isolated from the leaves of *Lophira lanceolata* for the first time. Preliminary phytochemical examination of the extract of the leaves of *Lophira lanceolata* revealed the presence of flavonoids, cardiac glycosides, anthraquinone, tannins, saponins and free reducing sugar while alkaloids are absent.

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