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Characterisation of 7-*O*-methylcupressuflavone, a biflavonoid from the leaves of *Lophira lanceolata*

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Abstract
The acetone extract of the leaves of *Lophira lanceolata* (Ochnaceae) was subjected to extensive column and thin-layer chromatography on silica. This yielded a biflavonoid identified as 7-*O*-methylcupressuflavone. The structure was elucidated using spectroscopic techniques, - UV, IR, NMR (2-D) and MS (Electrospray Ionisation – ESI). Preparation of methylated and acetylated derivatives was carried out, for confirmation. Spectroscopic data obtained are consistent with literature values for 7-*O*-methylcupressuflavone.

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

Introduction

Lophira lanceolata Tiegh ex Keay (family Ochnaceae) is a small to medium-sized tree, widely distributed in the Sudano-Guinean savanna zone from Senegal through the Central African Republic and northern DR Congo to Uganda. Its common names include red oak, red ironwood, and méni oil tree. Méni oil extracted from the seed has cosmetic and medicinal uses. For instance rubbing the skin with the oil prevents dryness. Méni oil is used, in traditional medicine, to treat dermatosis, toothache and muscular tiredness. In Mali pounded roots, mixed with flour are used to treat constipation. Also the root decoction is used to treat menstrual pain, intestinal troubles and malaria. In southern Nigeria the root bark is a remedy for yellow fever. The bark is also used to treat fevers and gastro-intestinal problems. Decoctions of the young red leaves are also employed in the treatment of headache, hypertension and syphilis (Mapongmetsem, 2007)

A phytochemical study of the bark has revealed presence of several flavonoids with some antibacterial and antiviral activity. They include the biflavonoids lophirones A-J, and isombamichalcone as well as the tetraflavonoid lanceochalcone. The wood contains the nitrile glycoside esters lanceolin A and B, while the leaves contain lanceolatin A and B and in addition the benzoyl glycoside lanceoloside A and the prenylated

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isoflavone lanceolone. The presence of benzamide has been reported in the root bark (Ewola-Tih *et al.,* 1994; Pegnyemb *et al*., 1994; Mapongmetsem, 2007). Compounds which have been isolated from the leaves of *Lophira lanceolata* include: flavonol, isoflavone and flavanol (Ghogomu-Tih *et al.,* 1994); lophiraic acid and its derivatives; lophirone A and its derivatives (Akira *et al.,* 1991). Of recent is the reported isolation of a biflavonoid, cupressuflavone (Sani *et al.,* 2010). The present work describes the isolation and characterization of a closely related compound, 7-*O*-methylcupressuflavone.

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. The dried, ground leaf 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, labelled extract 'A'.

Preliminary phytochemical screening. The presence of various classes of plant constituents (alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, tannins, and free reducing sugar) were tested using small portions of the extract. The tests were carried out in accordance with standard procedures (Markham, 1982; Evans, 1989).

Isolation. 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 (AGC), 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 "ALK2".

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 'ALK2' was methylated with potassium carbonate and dimethyl sulphate in dry acetone. The mixture was refluxed for 8 hrs. After work up the product, coded 'AM2'crystallized from $CHCl_3$ as colourless needles. Another portion of 'ALK2' was acetylated with pyridine and acetic anhydride. The acetate, after usual work up crystallized from $CHCl_3$-MeOH as colourless needles which was coded 'AT2'.

Results

The acetone extract was extracted with various organic solvents to afford a dark brown mass (23.8g). The mass gave dark green colour with alcoholic ferric chloride, orange colour with Mg-HCl and red colour with Zn-HCl. This clearly indicates that this fraction contain flavone nucleus. Other tests revealed the presence of anthraquinones, cardiac glycosides, saponins, tannins, and free reducing sugar while alkaloids were not detected.

Discussion

Cupressuflavone and 7-*O*-methylcupressuflavone showed very similar R_f values hence Sephadex LH 20 was used to separate them. 7-*O*-methyl cupressuflavone with five free phenolic hydroxyl groups

showed an R_f of 0.32 (BPF). This is only slightly higher than that of cupressuflavone which has four hydroxyl groups earlier reported with R_f of 0.30 (Sani *et al*., 2010). The small difference in the R_f values in BPF of cupressuflavone and 7-*O*-methyl cupressuflavone may however, be explained by the relative departure from planarity of the latter with subsequent variations in the magnitude of the conjugative effect. It could also be attributed to slightly lower polarity of 7-*O*-cupressuflavone.

^{1}H NMR data of ALK2 in DMSO

Position	δ (ppm)
H-3	6.797s
H-6	6.467d (2.2)
H-8	---------
H-2’	7.517dd (8.7/2.4)
H-3’	
H-5’	6.765d (8.7)
H-6’	7.517d (8.7/2.4)
H-3’’	6.797s
H-6’’	6.467s
H-8’’	------
H-2’’’, 6’’’	7.517d (8.8)
H-3’’’,5’’’	7.488d (8.8)
OH-4’, 4’’’	12.888s
OH-5, 5’’’	13.161s
OH-7 ,7’’’	
7-OMe	3.898s

^{13}C NMR data of ALK2 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 (ALK2) Molecular ion peak M^{+1}

354.09 , 361.02 , 378.97 , 419.99 , 450.97 , 459.44 , 476.98 , 478.97*(Molecular ion) , 480.05 , 510.72 , 537.92 , 538.75 , 551.53 , 569.54 , 597.62 , 629.44 , 657.48 , 683.60 , 685.62 , 707.55 , 727.65 , 729.62 , 731.63 , 751.61 , 771.63 , 775.61 , 771.63 , 775.59 , 805.42 , 815.60 , 817.63 , 819.58 , 828.56 , 859.64 , 861.68 , 863.62 , 871.59 , 903.66 , 905.67 , 907.71 , 915.63 , 947.69 , 950.73 , 952.70 , 991.66 , 993.70

Surprisingly, the ^{1}H NMR spectrum of the isolated product showed doublets at 6.726 (J = 8.6 Hz, 1H) and 6.765 (J = 1.9 Hz, 1H), and a doublets at 7.517 (J = 1.9 Hz, 1H) and 7.488 (J = 1.9, 8.6 Hz, 1H) indicating that ring-B was unchanged and fused.

COMPOUND ALK2: 7-*O*-METHYL CUPRESSUFLAVONE

[I-4’, II-4’, I-5, II-5- II-7 pentahydroxy I-7–*O*-methyl (I-8,II8) – Biflavone]

Yellow amorphous powder TLC R_f (1) 0.32, m.p. 179 – 180^{o}C
Elemental analysis; found: 66.91%C, 3.3%H. Molecular formula: $C_{31}H_{20}O_{10}$

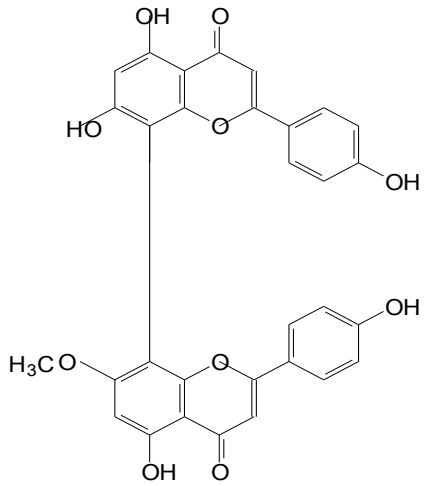
EI-MS *m/z* (rel. int.): 537 [M^-].

UV λ_{max} MeOH nm: 273, 326; +$AlCl_3$: 282, 303; +$AlCl_3$/HCl: 274, 301sh, 347, 380sh; +CH_3ONa: 288, 398; +CH_3COONa : 270, 287sh, 350; +CH_3COONa/H_3BO_3 279, 331.

IR (KBr) $cm^{-1:}$ 3444, 1648, 1610, 1588, 1546, 1508, 1192, 1145, 1070, 835; 3444, (OH), broad band around: and 1648 (C=O); 3444 = OH; α,β-unsaturated C=O (1646); and aromatic (1610 and 1588)

Methyl derivative: m.p. 182
Acetylated derivative: m.p. 187-188

7-O-methylcupressuflavone [I-4’,II-4’,I-5,II-5-,II-7- pentahydroxy -I-7–0-methyl (I-8,II8)–biflavone]



The structure of 7-*O*-methylcupressuflavone in *Lophira lanceolata* was determined by a combination of TLC, UV, IR, MS and NMR.

Differences in adsorption affinity are expected to occur with increasing methylation/ acetylation. As such fully methylated biflavones involving various modes of interflavonyl linkages were compared and found to show sizeable differences in R_f values. This aided the process of characterization. The different shades of the spots of these fully methylated derivatives in U.V. light (BPF) was also found to be helpful in their identification (Markham, 1982). The UV data indicated various ranges within the limit of biflavonoids.

The (M^+) MS for this compound 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, for (OH), and a broad band around 1648 for (C=O). From the MS data, seven compounds can be suggested to have the same mass and reactions; these are $C_{30}H_{18}O_{10}$, $C_{31}H_{22}O_9$, $C_{32}H_{26}O_8$, $C_{33}H_{30}O_7$, $C_{34}H_{34}O_6$, $C_{35}H_{38}O_5$, and $C_{36}H_{42}O_4$.

On spectral analysis of the ^{13}C and ^1H NMR of ALK2 it became clear that the only possible compound that could fit the data 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 presence of peak at higher field of δ 3.8ppm indicates the presence of a methoxy (-OCH_3) proton at carbon 7.

The isolation of this biflavone was carried out from the acetone extract by column chromatography using silica gel to give compound 'C1'. This was purified by re-chromatography on Sephadex LH-20 column (MeOH) to give ALK2. The position of the methoxy group was elucidated by ^{1}H NMR.

From the acetone extract of the leaves of *Lophira lanceolata*, a biflavone, unknown earlier in this species, has been isolated and identified as 7-*O*-methylcupressuflavone (ALK2). The structure was elucidated on the basis of TLC, UV, IR, ESI-MS and by comparison with the literature data (Geiger and Markham, 1996; Krauze-Baranowska *et al.*, 1999; Markham, 1982; Wollenweber *et al.*, 1998). The values are in agreement with values of chemical shifts of parallel carbon C-8/C-8" of 7-*O*-methylcupressuflavone (Krauze-Baranowska *et al.*, 1999). According to (Geiger and Markham 1996) the H-6 and H-8 signals in ^{1}H NMR spectrum of compound ALK2 were relatively downfield shifted, which suggested the methylation in position C-7 of this biflavone. The structure of compound ALK2 was established as 7-*O*-

methyl cupressuflavone by ^{1}H spectra in the family Ochnaceae.

Conclusion

Phytochemical examination of the extract of the leaves of *Lophira lanceolata* indicated the presence of flavonoids, cardiac glycosides, anthraquinones, tannins, saponins and free reducing sugar, while alkaloids are absent. Furthermore, the compound 7-*O*-methylcupresuflavone was isolated from the leaves of *Lophira lanceolata* for the first time.

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