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Flavonoids of *Ochna afzelii*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Fractionation of the methanolic extract of *Ochna afzelii* stem bark has resulted in the isolation of two biflavonoids afzelones A and B along with five known flavonoids, calodenins A and B, afzelone C, 4′,5-dimethoxy-6,7-methylenedioxyisoflavone, 4′,5,7-trimethoxyisoflavone and the glucoside lanceoloside A. Their structures were determined using spectroscopic and chemical methods.

Keywords: *Ochna afzelii*; Ochnaceae; Biflavonoids; Afzelones A and B; NMR spectroscopy

1. Introduction

Preliminary phytochemical studies on the Ochnaceae members in Cameroon, especially those of *Lophira* and *Ochna*, have revealed the occurrence of constituents representing biflavonoids of a new structural type (Ghogomu et al., 1987, 1989; Tih et al., 1990; Harborne, 1994). Some of the biflavonoids isolated from *Lophira alata* and the closely related species *L. lanceolata* have a variety of biological activities, such as antibacterial, anti-inflammatory and inhibitory activity against Epstein Barr virus (Murakami et al., 1992a,b). In our continuing search to discover and develop potential therapeutic agents from central Africa flora, *O. afzelii* R.Br ex Oliv. was collected near Yaounde (Cameroon) and phytochemically investigated. *O. afzelii* is a small tree which is widely found in the Central African forest and locally used in the treatment of jaundice, toothache, female infertility, menstrual complaints, lumbago and dysentery (Bouquet, 1969). In this paper we describe the isolation and structural elucidation of two new biflavonoids, afzelones A (**1**) and B (**2**) along with five known flavonoids, calodenins A (**3**) and B (**4**), afzelone C (**5**), 4′,5-dimethoxy-6,7-methylenedioxyisoflavone (**6**), 4′,5,7-trimethoxy-isoflavone (**7**) and the glucoside lanceoloside A (**8**).

2. Results and discussion

The stem bark of *O. afzelii* was ground and extracted with MeOH. Fractionation of the resulting dried residue, from the methanol solubles, was achieved by repeated column chromatography over silica gel with several eluting systems and final purification on Sephadex LH-20 column (MeOH), yielded eight compounds. Six of them were identified as calodenins A (**3**) and B (**4**) (Messanga et al., 1994), afzelone C (**5**) which had been previously isolated from *Brakenridgea zanguebarica* and described as an orange pigment with no trivial name (Drewes et al., 1984), 4′,5-dimethoxy-6,7-methylenedioxyisoflavone (**6**) (El-Emary et al., 1980), 4′,5,7-trimethoxy-isoflavone (**7**) (Moreira et al., 1994) and lanceoloside A (**8**) (Pegnyemb et al., 1998). The last two compounds were identified as afzelones A (**1**) and B (**2**) on the basis of the evidence outlined below.

Afzelone A (**1**) was obtained as an amorphous pale yellow solid and found to have the formula $C_{31}H_{24}O_9$ by HR MS, implying 20 unsaturated sites. Its IR spectrum showed absorption bands typical of OH groups

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(3320 cm^{-1}), conjugated carbonyl groups (1631 cm^{-1}), aromatic rings and conjugated double bonds (1540 cm^{-1}). Peracetylation (AC_2O/Pyr) of **1** gave an amorphous solid **1a** ($C_{39}H_{32}O_{13}$, $[M]^{+\bullet}$ m/z at 708), the ^{1}H-NMR spectrum of which had four sharp singlets at δ 2.32, 2.28, 2.20 and 2.18 (each 3H) assigned to four acetyl groups. Since the IR spectrum of **1a** had no residual OH absorption bands, it was deduced that **1** had four hydroxyl groups. The ^{13}C-NMR spectrum of **1** had signals for all 31 carbon atoms in the molecular formula, including signals for two carbonyl groups (δ_c 202.1 and 197.7) 12 quaternary sp^2 carbons with seven linked to an oxygen atom, 12 tertiary sp^2 carbons and five sp^3 carbons (Table 1). The ^{1}H-NMR spectrum displayed 12 aromatic protons and eight protons bound to sp^3 type carbon atoms (three CH, one CH_2 and one OCH_3). From detailed analysis of ^{1}H–^{1}H COSY NMR experiment, they were distributed into two 1,4-disubstituted, one 1,2,4-trisubstituted and one penta-substituted aromatic rings, whereas the aliphatic protons formed a singlet at δ_H 3.78 (3H) assigned to a MeO group, an aliphatic AB system at δ_H 5.31 and 5.96 and an ABX spin system at δ_H 3.22 (*dd*, J = 17.1, 13.1 Hz), δ_H 2.72 (*dd*, J = 17.1, 3.0 Hz) and 5.46 (*dd*, J = 13.1, 3.0 Hz). The long-range connectivities observed in the HMBC spectrum enabled the connection between these substructures with the aromatic rings as shown in structure **1** (Table 1). Carbonyl carbon atom c_1 (δ_c 197.7) was correlated to protons at δ_H 3.22, 2.72 (α_1) and 5.46 (β_1). The latter proton was correlated to carbon atoms at δ_c 129.0 (C-2′ and C-6′ on A_1-ring). The carbonyl carbon at δ_c 202.1 (C-2) correlated with the protons at δ_H 5.31 (α_2) and 5.96 (β_2) and with the aromatic proton H-6 on the B_2-ring (δ_H 7.75) ppm assigned at c_2. Proton (β_2) showed cross peaks with carbons at δ_c 128.5 (C-2′ and C-6′ on A_2-ring). Moreover, long-range coupling through an ethereal oxygen atom was observed between the protons of the MeO group (δ_H 3.78) and carbon C-4′ (A_2-ring) confirming the location of the MeO group at C-4′ (A_2-ring) in **1**. High NOE difference values were observed between H-α_2 and H-6 (B_2-ring) (23%) and between H-β_2 and H-2′,6′ (A_2-ring) (6%), while lower NOE values were observed between H-α_2 and H-β_2 (3%). This indicated a *trans*-configuration of H-α_2 and H-β_2 and led to structure **1** for afzelone A (relative stereochemistry). As far as we are aware, afzelone A is the first

Table 1
^{13}C- and ^{1}H-NMR spectral data for afzelones A (**1**) and B (**2**) (acetone-d_6)

C no.	**1**				**2**			
	δC	δH	*m* (*J*)	HMBC with H	δC	δH	*m* (*J*)	HMBC with H
1′-A_1	130.4	–		α_1; β_1; 3′,5′-A_1	130.7	–		α_1; 3′,5′-A_1
2′,6′-A_1	129.0	7.39	*m* 8.6	β_1; 2′,6′-A_1	129.0	7.37	*m* 8.5	2′,6′-A_1
3′,5′-A_1	116.1	6.90	*m* 8.6	3′,5′-A_1	116.1	6.87	*m* 8.5	3′,5′-A_1
4′-A_1	158.7	–		2′,6′-A_1	158.7	–		2′,6′,3′,5′-A_1
α_1	43.2	3.22	*dd* 17.1, 13.1	–	43.4	3.14	*dd* 17.2, 13.1	–
		2.72	*dd* 17.1, 3.0	–		2.63	*dd* 17.2, 2.9	–
β_1	80.2	5.46	*dd* 13.1, 3.0	α_1; 2′,6′-A_1	79.9	5.36	*dd* 13.1, 2.9	α_1; 2′,6′-A_1
c_1	197.7	–		α_1; β_1; 5-B_1	197.3	–		α_1; 5-B_1
1-B_1	103.9	–		α_1; 5-B_1	103.1	–		α_1; 5-B_1
2-B_1	159.8	–		α_2	163.0	–		α_2
3-B_1	106.4	–		α_2; β_2; 5-B_1	104.1	–		α_2; 5-B_1
4-B_1	169.4	–		α_2; β_2; 5-B_1	165.2	–		α_2; 5-B_1
5-B_1	90.9	6.08	*s*	–	95.2	5.88	*s*	–
6-B_1	167.8	–		5-B_1	163.0	–		5-B_1
1′-A_2	132.6	–		α_2; β_2; 3′,5′-A_2	130.5	–		α_2; β_2 3′,5′-A_2
2′,6′-A_2	128.5	7.35	*m* 8.6	β_2; 2′,6′-A_2	129.9	7.31	*m* 8.6	2′,6′-A_2
3′,5′-A_2	115.0	6.94	*m* 8.6	3′,5′-A_2	115.6	6.75	*m* 8.6	3′,5′-A_2
4′-A_2	161.1	–		2′,6′-A_2; OMe	158.4	–		2′,6′, 3′,5′-A_2
α_2	54.1	5.31	*d* 5.7	β_2	49.1	4.68	*d* 12.4	–
β_2	91.1	5.96	*d* 5.7	α_2; 2′,6′-A_2	83.2	5.96	*d* 12.4	α_2; 2′,6′-A_2
c_2	202.1	–		α_2; β_2; 6-BA_2	190.8	–		α_2; β_2; 6-B_2
1- B_2	113.5	–		3-B_2; 5-B_2	115.0	–		3-B_2; 5-B_2
2- B_2	165.6	–		3-B_2;6-B_2	164.5	–		3-B_2; 6-B_2
3- B_2	103.6	6.37	*d* 2.3	5-B_2	103.6	6.41	*d* 2.3	5-B_2
4- B_2	166.6	–		3-B_2; 6-B_2	164.9	–		5-B_2; 6-B_2
5- B_2	109.1	6.39	*dd* 9.0, 2.3	3-B_2	111.1	6.59	*dd* 8.6, 2.3	3-B_2
6- B_2	134.4	7.75	*d* 9.0	–	130.0	7.78	*d* 8.6	–
OMe	55.6	3.78	*s*	–	–	–		–
OH-2B_1	–	12.70	*s*	–	–	12.58	*s*	–
OH-2B_2	–	12.40	*s*	–	–	–		–

example of a biflavonoid isolated from a member of Ochnaceae family bearing a tetrahydrofuran ring and the six-membered heterocyclic ring on the same aromatic ring.

Afzelone B (**2**) was obtained as an amorphous yellow solid and found to be $C_{30}H_{22}O_9$ by HR MS. Evidence that **2** had five hydroxyl groups came from acetylation (AC_2O/Pyr), which gave an amorphous solid **2a** ($C_{40}H_{32}O_{14}$, $[M]^{+\bullet}$ at m/z 736); its ^{1}H-NMR spectrum had five sharp singlets at δ 2.34, 2.33, 2.29, 2.28 and 2.22 (each 3H) assigned to the five acetyl groups. From the 1D and 2D ^{1}H-NMR spectra of **2** (Table 1) it was established that 22 protons were located on four aromatic rings: two 1,4-disubstituted, one 1,2,4-trisubstituted and one pentasubstituted. Additionally the spectrum of **2** depicted an aliphatic AB system with *trans*-disposition at δ_H 4.68 and 5.96 ($J_{AB}=12.4$ Hz) and the ABX spin system at δ_H 3.14 (*dd*, $J=17.2$, 13.1 Hz), δ_H 2.63 (*dd*, $J=17.2$, 2.9 Hz) and 5.36 (*dd*, $J=13.1$, 2.9 Hz). The C–H connectivities in each of the aromatic rings were clearly assigned from the ^{1}H-detected heteronuclear multiple bond correlation spectrum (HMBC). This spectrum showed the following significant C–H connectivities through two or three bonds: the carbonyl carbon atom c_1 (δ_c 197.3) showed correlations with H-α_1 (δ_H 3.14 and 2.63) and with the proton at δ_H 5.88 (5-position of B_1-ring). Correlations were also depicted between H-β_1 (δ_H 5.36) and both C-2′ and C-6′ on A_1-ring (δ_c 129.0), between C-β_2 (δ_c 83.2) and both H-α_2 (δ_H 4.68) and H-2′ and H-6′ on A_2-ring (δ_H 7.31), and between C-α_2 (δ_c 49.1) and H-5 on B_1-ring (δ_H 5.88). Other C–H connectivities provided evidence for the structure of **2**, named afzelone B, and allowed assignments of the ^{13}C-NMR resonances (Table 1). The large coupling constant ($J=12.4$ Hz) between H-α_2 and H-β_2 observed in the ^{1}H-NMR spectrum implies their *trans*-diaxial disposition as shown in **2** (relative stereochemistry).

3. Experimental

3.1. General

IR spectra were obtained using KBr disks and recorded on a Nicolet Impact 400 D. UV spectra were obtained on an Uvikon 930 Kontron instrument and optical rotations were measured on a Perkin-Elmer 341 polarimeter. NMR ^{1}H, 300 MHz and ^{13}C, 75 MHz spectra were performed in Me_2CO-d_6 solution on a Bruker AC 300 instrument using TMS as internal standard. For HMBC spectra, the delay was optimized for $J_{CH}\approx 7$ Hz. The EI and CI mass spectra were recorded on a Nermag R 10-10 apparatus (using NH_3 for the CI mass spectra) and the HR ESI mass spectra on an Applied Biosystems API Q-STAR PULSAR i.

3.2. Plant material

O. afzelii R. Br. ex Oliv. was collected at Nkol-Afamba (Yaoundé, Center province of Cameroon) in April 1997. A voucher specimen (No. 23456: SRF/CAM) is deposited at the National herbarium in Yaounde (Cameroon).

3.3. Extraction and isolation

The dried bark of *O. afzelii* was extracted mechanically with MeOH at room temperature. After filtration and evaporation at normal pressure, the crude material (400 g) was subjected to silica gel chromatography, and eluted with the following gradient: CH_2Cl_2/MeOH (20/1; 10/1; 5/1) and finally MeOH. The fractions collected were combined into 11 major fractions (OA_1–OA_{11}) on the basis of TLC composition. Fraction OA_2 (4.3 g) was subjected to repeated cc on silica gel eluted with CH_2Cl_2/MeOH (50/1) to yield 4′,5-dimethoxy-6,7-methylenedioxyisoflavone (**6**, 30 mg) and 4′,5,7-trimethoxyisoflavone (**7**, 52 mg). Fraction OA_3 (1.2 g) crystallized in acetone and was applied to a Sephadex LH 20 column with MeOH as eluant, to give lanceoloside A (**8**) (22 mg). Fraction OA_5 (1.6 g) was first subjected to silica gel cc eluted with CH_2Cl_2/MeOH (15/1) yielding calodenins A (**3**) and B (**4**) and crude afzelone C (**5**) which was further purified over Sephadex LH 20 (MeOH) to yield pure afzelone C (9 mg). Fraction OA_6 (2.1 g) was further fractionated by cc (Si gel CH_2Cl_2/MeOH 10/1) into six sub-fractions. The sixth sub-fraction was purified by another cc step on silica gel with the same solvent system as before to give afzelones A (220 mg) and B (29 mg).

3.4. Afzelone A (*1*) $C_{31}H_{24}O_9$

Amorphous pale yellow solid ; $[\alpha]_D^{25}$ +193° (Me_2CO, 0.4). UV (EtOH) λ_{max} (log ϵ) 202 (4.67) 220 (4.66) 228 (4.65) 290 (4.52) 326 (sh 4.16). CIMS (NH_3) m/z 558 $[M+NH_4]^+$, 541 $[M+H]^+$. EIMS (110°, 70 eV) m/z (%): 540 (0.6, $M^{+\bullet}$), 543 (25), 448 (2), 431 (75), 404 (18), 395 (0.5), 388 (11), 313 (2), 273 (17), 213 (84), 151 (8), 131 (100), 121 (4), 110 (53), 82 (3). HR ESI MS m/z 5410.1492 $[M+H]^+$ (calc. for $C_{31}H_{25}O_9$: 541.1498). For ^{1}H and ^{13}C NMR spectra, see Table 1.

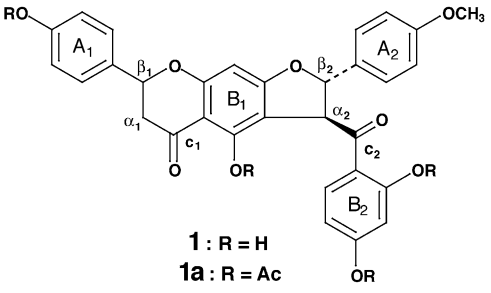
3.5. Afzelone A tetraacetate (*1a*) $C_{35}H_{32}O_{13}$

Afzelone A (10 mg) was dissolved in dry pyridine (2 ml) in a 5 ml round bottomed flask and AC_2O (2 ml) and left in the oven at 50 °C for 5 h, after which the solvent was removed under vacuum and the powder obtained purified over Sephadex LH 20 with MeOH as eluent to give afzelone A tetraacetate (**1a**), $C_{35}H_{32}O_{13}$ (5 mg). CIMS (NH_3) m/z 726 $[M+NH_4]^+$, 709 $[M+H]^+$.

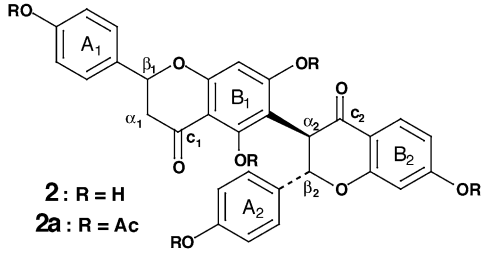
NMR ^{1}H (300 MHz) δ (ppm): 7.67 (2H, *m*, $J=8.6$ Hz, H-2′,6′ A_1), 7.06 (2H, *m*, $J=8.6$ Hz, H-3′,5′ A_1), 3.22 (1H, *dd*, $J=17.0$, 13.2 Hz, H-α_1), 2.72 (1H, *dd*, $J=17.0$, 3.0 Hz, H-α_1), 5.46 (1H, *dd*, $J=13.2$, 3.0 Hz, H-β_1), 6.15 (1H, *s*, H-5B_1), 7.35 (2H, *m*, $J=8.6$ Hz, H-2′,6′ A_2), 6.94 (2H, *m*, $J=8.6$ Hz, H-3′,5′ A_2), 5.47 (1H, *d*, $J=5.4$ Hz, H-α_2), 6.01 (1H, *dd*, $J=5.4$ Hz, H-β_2), 7.13 (1H, *d*, $J=2.3$ Hz, H-3 B_2), 7.36 (1H, *dd*, $J=8.8$, 2.3 Hz, H-5B_2), 8.24 (1H, *d*, $J=8.8$ Hz, H-6B_2), 3.79 (3H, *s*, OMe-A_2), 2.32 (3H, *s*, OAc), 2.28 (3H, *s*, OAc), 2.22 (3H, *s*, OAc), and 2.20 (3H, *s*, OAc).

3.6. Afzelone B (2) $C_{30}H_{22}O_9$

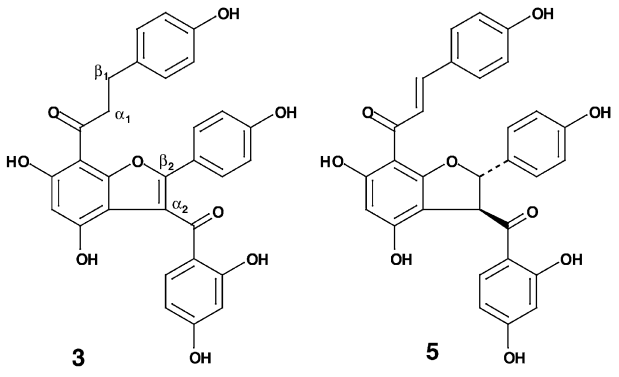
Amorphous pale yellow solid. $[\alpha]_D^{25}$ −19° (Me_2CO, 0.6). UV (EtOH) λ_{max} (log ϵ) 215 (4.70) 273 (4.20). CIMS (NH_3) *m/z* 545 $[M+NH_4]^+$, 528 $[M+H]^+$. EIMS (110°, 70 eV) *m/z* (%) 527 (0.5, $M^{+\bullet}$), 429 (8), 406 (84), 391 (65), 348 (21), 303 (20), 279 (18), 256 (75), 213 (12), 180 (33), 152 (35), 136 (22), 120 (29), 110 (83), 91 (4). HR ESI MS *m/z* 527.1343, $M^{+\bullet}$ (calc. for $C_{30}H_{23}O_9$, 527.1341). For ^{1}H and ^{13}C NMR spectra, see Table 1.



1 : R = H
1a : R = Ac



2 : R = H
2a : R = Ac



3 **5**

4 : E-α_1,β_1-dehydroderivative of **3**

3.7. Afzelone B pentaacetate (2a) $C_{40}H_{32}O_{14}$

The same procedure as for **1** was applied to **2** (10 mg) and its pentaacetate **2a**, $C_{40}H_{32}O_{14}$ (6 mg) was obtained. CIMS (NH_3) *m/z* 754 $[M+NH_4]^+$, 737 $[M+H]^+$. NMR ^{1}H (300 MHz) δ (ppm): 7.54 (2H, *m*, $J=8.5$ Hz, H-2′,6′ A_1), 7.19 (2H, *m*, $J=8.5$ Hz, H-3′,5′ A_1), 3.14 (1H, *dd*, $J=17.1$, 13.2 Hz, H-α_1), 2.63 (1H, *dd*, $J=17.1$, 2.8 Hz, H-α_1), 5.33 (1H, *dd*, $J=13.2$, 2.8 Hz, H-β_1), 6.23 (1H, *s*, H-5B_1), 7.48 (2H, *m*, $J=8.6$ Hz, H-2′,6′ A_2), 7.07 (2H, *m*, $J=8.6$ Hz, H-3′,5′ A_2), 4.72 (1H, *d*, $J=12.5$ Hz, H-α_2), 5.90 (1H, *d*, $J=12.5$ Hz, H-β_2), 6.74 (1H, *d*, $J=1.9$ Hz, H-3 B_2), 6.89 (1H, *dd*, $J=8.8$, 1.9 Hz, H-5B_2), 8.32 (1H, *d*, $J=8.8$ Hz, H-6B_2), 2.34 (3H, *s*, OAc), 2.33 (3H, *s*, OAc), 2.29 (3H, *s*, OAc), 2.28 (3H, *s*, OAc), and 2.22 (3H, *s*, OAc).

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