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Two biflavonoids from *Ouratea flava* stem bark

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Abstract

In a chemical investigation on the stem bark of *Ouratea flava*, two biflavonoids: 1-[3-(2,4-dihydroxy-benzoyl)-4,5,6-trihydroxy-2-(4-hydroxy-phenyl)-benzofuran-7-yl] -3-(4-hydroxy-phenyl) -propenone (flavumone A) and 3-(2,4-dihydroxy-benzoyl)-4-hydroxy-2,7-bis-(4-hydroxy-phenyl) -7,8- dihydro-furo[2,3-f]chromen-9-on (flavumone B) were isolated along with five known flavonoids. Their structures were established by various analyses including 2D-NMR spectroscopy.

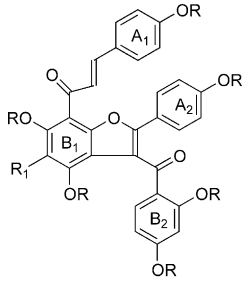
Keywords: *Ouratea flava*; Ochnaceae; Stem bark; Biflavonoids; Flavumones A and B; NMR data

1. Introduction

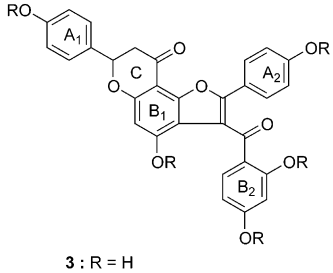
The genus *Ouratea* comprises 300 tropical species occuring mainly in South America (Heywood, 1978), and its extracts are used in folk medicine in cases of rheumatic and gastric distress. Previous investigations on the chemical composition of this genus have led to the isolation of several flavonoids (Velandia et al., 1998) and biflavonoids from the leaves (Moreira et al., 1999 ; Felicio et al., 2001) and from stem bark (Moreira et al., 1994). Some of biflavonoids isolated from *Ouratea spectabilis* have shown an inhibitory effect against lens aldose reductase. The increased activity of this enzyme has been implicated in the pathogenesis of many diabetic complications, such as cataract, retinopathy, and neuropathy (Felicio et al., 1995). In the course of our phytochemical investigations on Cameroonian medicinal plants, we studied here the constituents of *Ouratea flava*. In this paper, we report the isolation and structural elucidation of two new biflavonoids: flavumones A (**2**) and B (**3**).

2. Results and discussion

The air-dried stem bark of *Ouratea flava* was extracted with methanol. Constituents were separated by the combination of column chromatography on silica gel and Sephadex LH-20 gel. Final purification gave seven compounds, five of them were identified as calodenins B (**1**) and C (Messanga et al., 1994, 1998), lophirone A (Ghogomu et al., 1987), methoxyirilone (Messanga et al., 1998) and 4′,5-dimethoxy-6,7-methylenedioxyisoflavone (El-Emary et al., 1980).



1 : R = R_1 = H
2 : R = H, R_1 = OH
4 : R = Ac, R_1 = OAc



3 : R = H
5 : R = Ac

Compound **2**, named flavumone A was obtained as yellow crystals. In the CIMS of **2**, the pseudomolecular ion $[M+H]^+$ occurred at m/z 541 in agreement with the molecular formula $C_{30}H_{20}O_{10}$, indicating one oxygen atom more than that of calodenin B (**1**). The ^1H and ^{13}C NMR spectra signals showed a close analogy with those of **1**. The presence in the spectrum of **2** of two carbonyl carbon atoms (δc 190.6 and 195.7), two conjugated carbon–carbon bonds (δc 145.1, 123.0, 153.5 and 113.7), two *para*-disubstituted and one *ortho, para*-trisub-

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stituted aromatic rings as in the structure of **1** was evident. The main difference between ^{13}C NMR spectra of the two compounds, was the presence of a quaternary sp^2 carbon bearing an oxygen atom at δc 155.1 in the spectrum of **2**, instead of a tertiary sp^2 carbon at δc 99.7 in the spectrum of **1**. The absence of a singlet at δ_H 6.28 in the ^1H NMR spectrum of flavumone A suggested that **2** was a derivative of **1**, with an hydroxy group on the B_1-aromatic ring.

Structure **2** was confirmed from the analysis of its HMBC spectrum (acetone-d_6). The lower field carbonyl carbon signal at δc190.6 was correlated with protons at δ_H 8.29 (H-α_1) and 7.94 (H-β_1). The carbon atom at δc 145.1 was correlated to the protons of the *para*-disubstituted aromatic A_1-ring (7.74 ppm) forming a *para*-hydroxydehydrocinnamoyl group. The carbonyl carbon atom at δc 195.7 (c_2) showed ^3J correlation with the H-6 proton (7.76 ppm) at the aromatic B_2-ring and the carbon at 153.2 ppm (β_2) was correlated to the aromatic protons H-2′,6′ (7.63 ppm) of the *para*-disubstituted aromatic A_2-ring.

The angular position of the *para*-hydroxydehydrocinnamoyl group linked to the aromatic B_1-ring was determined after analysing of HMBC spectrum of **2** dissolved in DMSO-d_6. In this spectrum, the phenolic protons of B_1-ring appeared clearly and gave significant long-range correlations. The quaternary sp^2 carbon atom at δc 102.6 (C-1′, B_1-ring) which is linked to cinnamoyl group, was correlated only with the chelated phenolic hydroxyl (δ_H 13.37) whereas cross-peaks were observed between the signal of the sp^2 carbon at δc 155.1 and both phenolic hydroxyls.

Complete acetylation of **2** with acetic anhydride/pyridine afforded a hepta-acetate derivative **4** that analysed for $C_{44}H_{34}O_{17}$ (CIMS: $[M+H]^+$ at m/z 835) indicative of seven phenolic hydroxyls as expected. Based on the above evidence leads to structure **2** for flavumone A.

Compound **3** named flavumone B was isolated as an amorphous yellow solid. Its molecular formula was determined as $C_{30}H_{20}O_9$ by analysis of its higher resolution mass spectrum (m/z 524.0968). The IR spectrum of **3** showed absorption bands for a conjugated and H-bonded carbonyl group (ν_{max} 1649 cm^{-1}), an aromatic ring (ν_{max} 1600, 1570, 1560, 1515 cm^{-1}) and a hydroxyl group (ν_{max} 3370 and 3320 cm^{-1}). Peracetylation (Ac_2O/Py) of **3** gave an amorphous solid **5** ($C_{40}H_{30}O_{14}$, $[M+H]^+$ 735), the ^1H NMR spectrum of which had five sharp singlets at δ 2.31, 2.29, 2.22, 2.19 and 2.18 (each 3H) assigned to five acetyl groups. Since IR spectrum of **5** had no residual OH absorption bands, it was deduced that **3** had five hydroxyl groups. The ^1H NMR spectrum of **3** showed twelve aromatic protons and three protons of sp^3 type carbon atoms. From detailed analysis of ^1H–^1H COSY NMR, they were distributed into two *para*-substituted aromatic, one *ortho*, *para*-tri-substituted, one pentasubstituted aromatic rings, whereas the aliphatic protons formed an ABX spin system at 2.78 (*dd*, $J=17.4$; 2.8 Hz), 3.15 (*dd*, $J=17.4$; 13.0 Hz) and 5.55 (*dd*, $J=13.0$; 2.8 Hz).

The ^{13}C NMR spectrum on the other hand, showed 30 carbon signals including signals for two carbonyl groups (δc 188.2 and 197.1), 14 quaternary sp^2 carbons with eight bearing an oxygen atom, eight tertiary sp^2 carbons and three sp^3 carbons (Table 2).

The HMBC spectrum of **3** additionally indicated the following significant C–H connectivities through two or three bonds, between the aliphatic carbon (β_1, δc 80.9) and the two protons at 2,6-position of ring A_1 (δ_H 7.44), between the carbon atom at 154.8 ppm with protons at 2,6-position of ring A_2 (δ_H 7.60), carbonyl carbon c_2 (δc 197.1) showed correlation with the aromatic proton H-6′ on B_2-ring (δ_H 7.41). However an ambiguity remained concerning the respective position on the B_1-ring of the C-ring and its unique proton, leading either to an angular or linear total structure. This problem was solved by analysing the ^1H NMR spectrum of **3** dissolved in DMSO-d_6. In this spectrum the five phenolic protons of the molecule were clearly depicted among which only one was strongly chelated at 12.56 ppm suggesting that the C-ring had an angular orientation in **3**. Based on the above evidence, structure **3** is thus proposed for flavumone B.

3. Experimental

3.1. General

IR spectra were obtained using KBr discs. NMR ^1H, 400 MHz and ^{13}C, 100 MHz spectra were performed in Me_2CO-d_6 solution on a bruker WM 400 instrument using TMS as internal standard. For the HMBC spectra, the delay was 70 ms ($J_{CH}\approx7$ Hz).

3.2. Plant material

The stem bark of *Ouratea flava*, Schum and Thon. was collected in Eloumdem mount (Yaounde, Center province of Cameroon) in July 2000. A voucher specimen (N° 27056/SRF/CAM) is deposited at the National Herbarium in Yaounde, Cameroon.

3.3. Extraction and isolation

Air-dried stem bark of *O. flava* (2.5 kg) was extracted mechanically for 48 h with MeOH at room temperature and afterwards filtered and concentrated under vacuum. The crude MeOH extract (120 g) was first subjected to column chromatography on silica gel and elution with CH_2Cl_2/MeOH mixture of increasing polarity to give six fractions (OU_1 to OU_6) on the basis of TLC com-

position. Fraction OU_2 (9.6 g) was subjected to repeated CC on silica gel eluted with CH_2Cl_2/MeOH (50/1) to yield methoxyirilone (100 mg) and 4′,5-dimethoxy-6,7-methylenedioxyisoflavone (70 mg). Fraction OU_3 (6.5 g) was first chromatographed on a silica gel column (CH_2Cl_2/MeOH:20/1) to give crude fractions OU_3C and OU_3F which were further purified using the same procedure as before to yield pure lophirone A (100 mg) and calodenin C (25 mg). Fraction OU_4 (2.3 g) was purified by CC with the solvent mixture CH_2Cl_2/MeOH (10/1). Further purification was realised by gel permeation chromatography over Sephadex LH-20 with MeOH to give flavumone A (**2**) (20 mg). Fraction OU_5 (1.7 g) was first chromatographed over silica gel (CH_2Cl_2/MeOH 15/1) yielding calodenin B (**1**) (30 mg) and crude flavumone B which was further purified over Sephadex LH-20 (MeOH) to yield pure flavumone B (**3**) (15 mg).

3.4. Flavumone A (2), $C_{30}H_{20}O_{10}$

Yellow crystals, mp 240–241° (Me_2CO), CIMS (NH_3) *m/z*: 558 $[M+NH_4]^+$, 541 $[M+H]^+$. EIMS (110°, 70 eV) *m/z* (%): 540 (13, $M^{+\cdot}$), 310 (30), 284 (12), 183 (29), 165 (75), 137 (20), 81 (48), 69 (77), 57 (100). HREIMS *m/z*: 540.10565 $M^{+\cdot}$ (calc. For $C_{30}H_{20}O_{10}$; 540.47380). ^1H and ^{13}C NMR: Tables 1 and 2.

Table 1
^1H NMR data for compounds **2** and **3** (400 MHz, acetone-d_6, TMS)

	2			**2**[a]			**3**			**3**[a]		
	δH (ppm)	*m*	*J* (Hz)	δH (ppm)	*m*	*J* (Hz)	δH (ppm)	*m*	*J* (Hz)	δH (ppm)	*m*	*J* (Hz)
$1A_1$	–			–			–			–		
$2A_1$	7.74	*m*	8.5	7.67	*m*	8.5	7.44	*m*	8.8	7.32	*m*	8.6
$3A_1$	6.99	*m*	8.5	6.89	*m*	8.5	6.89	*m*	8.8	6.73	*m*	8.6
$4A_1$	–			–			–			–		
$5A_1$	6.99	*m*	8.5	6.89	*m*	8.5	6.89	*m*	8.8	6.73	*m*	8.6
$6A_1$	7.74	*m*	8.5	7.67	*m*	8.5	7.44	*m*	8.8	7.32	*m*	8.6
α_1	8.28	*d*	15.4	8.17	*d*	15.4	3.15	*dd*	17.4; 13.0	3.28	*dd*	17.4; 13.0
							2.78	*dd*	17.4; 2.8	2.69	*dd*	17.4; 2.8
β_1	7.93	*d*	15.4	7.85	*d*	15.4	5.55	*dd*	13.0; 2.8	5.47	*dd*	13.0; 2.8
c_1	–			–			–			–		
$1'B_1$	–			–			–			–		
$2'B_1$	–			–			–			–		
$3'B_1$	–			–			–			–		
$4'B_1$	–			–			–			–		
$5'B_1$	–			–			6.34	*s*		5.98	*s*	
$6'B_1$	–			–						–		
$1A_2$	–			–						–		
$2A_2$	7.63	*m*	8.5	7.52	*m*	8.5	7.60	*m*	8.9	7.42	*m*	8.8
$3A_2$	6.92	*m*	8.5	6.86	*m*	8.5	6.91	*m*	8.9	6.83	*m*	8.8
$4A_2$	–			–			–			–		
$5A_2$	6.92	*m*	8.5	6.86	*m*	8.5	6.91	*m*	8.9	6.83	*m*	8.8
$6A_2$	7.63	*m*	8.5	7.52	*m*	8.5	7.60	*m*	8.9	7.42	*m*	8.8
α_2	–			–			–			–		
β_2	–			–			–			–		
c_2	–			–			–			–		
$1'B_2$	–			–			–			–		
$2'B_2$	–			–			–			–		
$3'B_2$	6.40	*d*	2.3	6.33	*d*	2.3	6.38	*d*	2.3	6.30	*d*	2.3
$4'B_2$	–			–			–			–		
$5'B_2$	6.25	*dd*	8.8; 2.3	6.31	*dd*	8.8; 2.3	6.23	*dd*	8.8; 2.3	6.18	*dd*	8.8; 2.3
$6'B_2$	7.46	*d*	8.8	7.32	*d*	8.8	7.41	*d*	8.8	7.30	*d*	8.8
OH ($2'B_1$)				13.37	*s*					–		
OH ($2'B_2$)				12.38	*s*					12.56	*s*	
OH ($4'B_1$)				11.31	*s*					10.77	*s*	
OH ($4'B_2$)				10.85	*s*					10.58	*s*	
OH ($4A_1$)				9.20	*s*					9.60	*s*	
OH ($4A_2$)				9.91	*s*					9.99	*s*	
OH ($3'B_2$)				9.95	*s*							

Acetone-d_6.
[a] DMSO-d_6.

Table 2
^{13}C NMR data and HMBC correlations for compound **2** and **3** (100 MHz, acetone-d_6, TMS)

	2		**3**	
	δC (ppm)	HMBC (protons)	δC (ppm)	HMBC (protons)
$1A_1$	125.7	$2,6A_1$	131.0	α_1, β_1, $3,5A_1$
$2A_1$	131.6	$3A_1$	129.1	β_1, $6A_1$
$3A_1$	116.1	$2A_1$	116.1	$5A_1$
$4A_1$	161.1	$3,5A_1$	158.6	$2,6A_1$
$5A_1$	116.1	$6A_1$	116.1	$3A_1$
$6A_1$	131.6	$5A_1$	129.1	β_1, $2A_1$
α_1	123.0	β_1	45.0	–
β_1	145.1	α_1; $2,6A_1$	80.9	α_1; $2,6A_1$
c_1	190.6	α_1, β_1	188.2	α_1, β_1, $5'B_1$
$1'B_1$	101.5	–	115.4	α_1, $5'B_1$
$2'B_1$	158.7	–	156.3	–
$3'B_1$	115.3	–	103.3	$5'B_1$
$4'B_1$	167.0	–	163.5	$5'B_1$
$5'B_1$	154.9	–	99.4	–
$6'B_1$	155.0	–	157.7	$5'B_1$
$1A_2$	121.7	$2,6A_2$	121.7	$3,5A_2$
$2A_2$	129.2	$3A_2$	129.6	$6A_2$
$3A_2$	116.7	$2A_2$	116.6	$5A_2$
$4A_2$	159.5	$3,5A_2$	159.5	$2,6A_2$
$5A_2$	116.7	$6A_2$	116.6	$3A_2$
$6A_2$	129.2	$5A_2$	129.6	$2A_2$
α_2	113.6	–	113.2	–
β_2	153.2	$2,6A_2$	154.8	$2,6A_2$
c_2	195.7	$6'B_2$	197.1	$6'B_2$
$1'B_2$	115.9	$5',6'B_2$	116.1	$3',5'B_2$
$2'B_2$	166.2	$3',6'B_2$	166.3	$3',6'B_2$
$3'B_2$	103.3	$5'B_2$	103.4	$6'B_2$
$4'B_2$	166.3	$3',5',6'B_2$	166.3	$3',6'B_2$
$5'B_2$	109.1	$3',6'B_2$	109.1	$3'B_2$
$6'B_2$	136.7	$5'B_2$	136.7	–

3.5. Flavumone A heptaacetate (**4**)

Flavumone 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° 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 flavumone A heptaacetate (**4**), $C_{44}H_{34}O_{17}$ (5 mg). CIMS (NH_3) m/z 852 $[M+NH_4]^+$, 835 $[M+H]^+$, NMR ^1H (400 MHz) δ (ppm) : 7.96 (2H, *m*, H-2,6A_1), 7.36 (2H, *m*, H-3,5A_1), 7.75 (1H, *d*, $J=15.7$ Hz, H-α_1), 7.93 (1H, *d*, $J=15.7$ Hz, H-β_1), 7.84 (2H, *m*, H-2,6A_2), 7.28 (2H, *m*, H-3,5A_2), 7.13 (1H, *d*, $J=2.4$ Hz, H-3′B_2), 6.99 (1H, *dd*, $J=8.8$; 2.4 Hz, H-5′B_2), 7.31 (1H, *d*, $J=8.8$ Hz, H-6′B_2), 2.41 (3H, *s*, OAc), 2.39 (3H, *s*, OAc), 2.27 (3H, *s*, OAc), 2.22 (3H, *s*, OAc), 2.21 (3H, *s*, OAc), 2.20 (3H, *s*, OAc), 2.18 (3H, *s*, OAc).

3.6. Flavumone B (**3**), $C_{30}H_{20}O_9$

Amorphous yellow solid. $[\alpha]_D^{25} = +29°$ (MeOH, *c* 0.5), CIMS (NH_3) m/z 542 $[M+NH_4]^+$, 525 $[M+H]^+$. TOF-MS m/z (%): 525.1 (100, $[M+H]^+$), 513.2 (32), 483.2 (42), 411.4 (13), 375.2 (34), 369.4 (19), 288.3 (22), 270.2 (15), 197.1 (36), 149.0 (14), 139.1 (19), 121.0 (15), 116.1 (11). HREIMS m/z: 524.0968 $M^{+\cdot}$ (calc. for $C_{30}H_{20}O_9$, 524.1106). ^1H and ^{13}C NMR: Tables 1 and 2.

3.7. Flavumone B pentaacetate (**5**)

The same procedure as for 2 was applied to 3 (10 mg) and its pentaacetate **5**, $C_{40}H_{30}O_{14}$ (5 mg) was obtained. CIMS (NH_3) m/z 752 $[M+NH_4]^+$, 735 $[M+H]^+$. NMR ^1H (400 MHz) δ (ppm) : 7.71 (2H, *m*, $J=8.8$ Hz, H-2,6A_1), 7.05 (2H, *m*, $J=8.8$ Hz, H-3,5A_1), 3.16 (1H, *dd*, $J=17.4$; 13.1Hz, H-α_1), 2.73 (1H, *dd*, $J=17.4$; 2.8 Hz, H-α_1), 5.58 (1H, *dd*, $J=13.1$; 2.8 Hz, H-β_1), 6.52 (1H, *s*, H-5′B_1), 7.74 (2H, *m*, $J=8.9$ Hz, H-2,6A_2), 7.14 (2H, *m*, $J=8.9$ Hz, H-3,5A_2), 6.92 (1H, *d*, $J=2.3$ Hz, H-3′B_2), 6.82 (1H, *dd*, $J=8.8$; 2.3 Hz, H-5′B_2), 7.81 (1H, *d*, $J=8.8$ Hz, H-6′B_2), 2.31 (3H, *s*, OAc), 2.29 (3H, *s*, OAc), 2.22 (3H, *s*, OAc), 2.19 (3H, *s*, OAc), 2.18 (3H, *s*, OAc).

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