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Three isoflav-3-enes and a 2-arylbenzofuran from the root bark of *Erythrina burttii*

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Abstract

From the root bark of *Erythrina burttii* three isoflav-3-enes, 7,4′-dihydroxy-2′-methoxy-6-(1″,1″-dimethylallyl)isoflav-3-ene (trivial name, burttinol-A), 4′-hydroxy-2′-methoxy-2″,2″-dimethylpyrano[5″,6″:8,7]isoflav-3-ene (trivial name, burttinol-B), 7,4′-dihydroxy-2′-methoxy-8-(3″,3″-dimethylallyl)isoflav-3-ene (trivial name, burttinol-C), and 2-arylbenzofuran, 6,4′-dihydroxy-2′-methoxy-5-(1″,1″-dimethylallyl)-2-arylbenzofuran (trivial name, burttinol-D) were isolated. In addition, the known compounds, abyssinone V-4′-methyl ether, bidwillol A, calopocarpin, erybraedin A, erythrabyssin II, isobavachalcone, phaseollidin and phaseollin were identified. The structures were determined on the basis of spectroscopic evidence. © 2002 Published by Elsevier Science Ltd.

Keywords: *Erythrina burttii*; Leguminosae; Root bark; Isoflav-3-enes; 2-Arylbenzofuran; Burttinol-A; Burttinol-B; Burttinol-C; Burttinol-D

1. Introduction

The root and stem bark of *Erythrina* species are widely used in traditional medicine for the treatment of microbial infections (Mitscher et al., 1987). Pterocarpans isolated from some of these species are mainly associated with the antimicrobial effects of these plants (Mitscher et al., 1987, 1988). In addition to pterocarpans, an isoflav-3-ene and a 2-arylbenzofuran isolated from the root bark of *Erythrina bidwillii* showed significant antimicrobial activity against oral bacteria (Iinuma et al., 1994).

In our interest on the phytochemistry of *Erythrina* species of Kenya, we have reported flavanones from the stem bark of *Erythrina burttii* (Yenesew et al., 1998b), isoflavones and isoflavanones from the stem bark of *Erythrina sacleuxii* (Yenesew et al., 1998a, 2000). We report here the isolation and characterization of three new isoflav-3-enes and a new 2-arylbenzofuran from the root bark of *Erythrina burttii* along with eight known flavonoids.

2. Results and discussion

The root bark of *Erythrina burttii* was extracted with acetone. The crude extract was subjected to flash column chromatography followed by preparative thin layer chromatography, which resulted in the isolation of twelve compounds. Four of these are new compounds.

Compound **1** was isolated as yellow oil. The UV (λ_{max} at 239, 328 nm), ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra of **1** suggested an isoflav-3-ene skeleton (Stevenson and Veitch, 1996; Fukai et al., 1996). The presence of two hydroxyl, a methoxyl and a 1,1-dimethylallyl substituents were also evident from the MS ($[M]^+$ at m/z 338), ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra.

Furthermore, the occurrence in the ^{1}H NMR spectrum of two aromatic singlets at δ 6.95 and 6.42, and an AXY spin system is consistent with oxygenation at C-7, C-2′ and C-4′, with the 1,1-dimethylallyl group being at C-6 or C-5′. In the HMBC spectrum, correlation was observed between the singlet at δ 6.95 and δ 121.1 (C-4). Hence the singlet at δ 6.95 was assigned to H-5 and the singlet at δ 6.42 to H-8. This observation is in agreement with the placement of the 1,1-dimethylallyl group at C-6

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(**1**) rather than C-5′. Confirmation of this was achieved from the NOESY spectrum, which showed nOe interaction between the methyl protons of the 1,1-dimethylallyl group with H-5 and OH-7, as well as from the HMBC spectrum where H-5 correlates with C-1″.

In the ^{1}H NMR spectrum, the AXY spin system (Table 1) was due to the B-ring protons (H-3′,-5′ and-6′) with hydroxyl and methoxyl groups being at C-2′ and C-4′. The chemical shift values of the carbon atoms (Table 2) in B-ring are in agreement with such oxygenation (Fukai et al., 1996). Finally, the position of the methoxyl group was fixed at C-2′ rather than C-4′ from the NOESY spectrum of this compound as well as from that of the di-acetate derivative (**1a**), which showed NOE

Table 1
^{1}H NMR data of isoflav-3-enes of *Erythrina burttii* (300 MHz, $CDCl_3$)

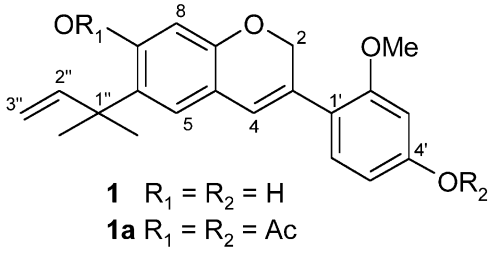
H	**1**	**1a**	**2**	**2a**	**3**
2	4.97 *s*	5.00 *s*	4.99 *s*	5.01 *s*	4.97 *s*
4	6.52 *s*	6.53 *s*	6.47 *s*	6.64 *s*	6.50 *s*
5	6.95 *s*	7.21 *s*	6.81 *d*	6.93 *d*	6.82 *d*
6			6.38 *d*	6.35 *d*	6.41 *d*
8	6.42 *s*	6.74 *s*			
3′	6.42 *d*	6.84 *d*	6.40 *d*	6.82 *d*	6.42 *d*
5′	6.41 *dd*	6.76 *dd*	6.38 *dd*	6.75 *dd*	6.41*dd*
6′	7.13 *d*	7.38 *d*	7.13 *d*	7.35 *d*	7.15 *d*
1″					3.41 *d*
2″	6.19 *dd*	5.95 *dd*			5.27 *t*
3″	5.33 *d*	5.03 *d*	5.59 *d*	5.71 *d*	
	5.28 *d*	4.93 *d*			
4″			6.65 *d*	6.61 *d*	
Me_2	1.43 *s*	1.41 *s*	1.43 *s*	1.40 *s*	1.74 *s*
					1.82 *s*
OMe	3.75 *s*	3.86 *s*	3.76 *s*	3.85 *s*	3.79 *s*
OH	5.98 *br s*				5.07*br s*
OAc		2.26 *s*		2.26 *s*	
		2.17 *s*			

J values: H-5/H-6 in **2, 2a** and **3** = 8.2 Hz; H-3′/H-5′ = 2.1 Hz; H-5′/H-6′ = 8.7 Hz; H-2″/H-3″ in **1, 1a** and **3** = 10.2, 18.1 Hz; H-1″/H-2″ in **3** = 6.9 Hz; H-3″/H-4″ in **2** and **2a** = 9.9 Hz.

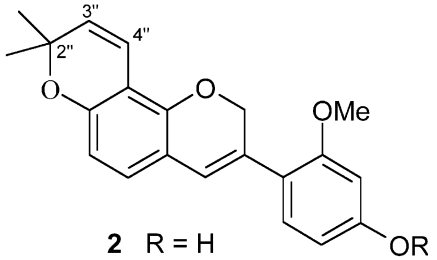
Table 2
^{13}C NMR data of isoflav-3-enes of *Erythrina burttii* (75 MHz, $CDCl_3$)

C	**1**[a]	**1a**	**2**[a]	**2a**	**3**
2	68.3	67.9	68.4	68.1	68.4
3	129.4[b]	129.0	128.9	128.9[b]	128.9
4	121.1	122.3	121.4	122.6	121. 8[b]
4a	116.8	121.1	117.0	117.0	117.1
5	124.5	125.5	126.5	127.2	125.0
6	125.4	132.7	109.3	109.5	108.7
7	155.0	152.5[b]	153.1	153.9	155.1
8	104.8	111.8	109.6	108.8	114.4
8a	153.3	149.5	149.0	149.3	151.9
1′	120.2	125.0	120.4	125.3	120.7
2′	158.3	158.3	158.3	158.2	158.4
3′	99.2	105.8	99.1	105.7	99.1
4′	157.0	152.3[b]	156.8	152.1	156.6
5′	107.3	109.5	107.3	109.5	107.3
6′	129.3[b]	131.5	129.4	129.8[b]	129.4
1″	39.7	39.8			22.4
2″	148.0	147.6	76.2	76.1	121.9[b]
3″	113.3	114.2	129.4	129.8[b]	134.5
4″			116.6	116.4	
Me_2	27.1	27.5	27.7	27.3	17.9
					25.8
OMe	55.3	55.5	55.3	55.5	55.4
Me<u>C</u>=O		168.9		168.9	
		168.7			
<u>Me</u>C=O		21.0		20.3	
		20.4			

[a] ^{13}C-Assignments were based on HMQC and HMBC experiments.
[b] Assignments in the same column are interchangeable.



1 $R_1 = R_2 = H$
1a $R_1 = R_2 = Ac$



2 R = H
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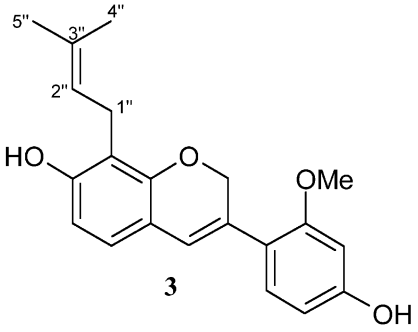

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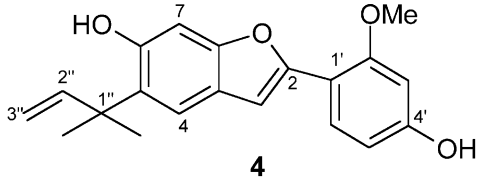

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interaction of the methoxyl protons with the methylene protons at C-2 and H-3′. The placement of the methoxyl group at C-2′ is consistent with a negative Gibb's test for compound **1** (King et al., 1957; Rao and Krupadanam, 1994; Fukai et al., 1996). The identity of compound **1** was confirmed by HMBC and HMQC experiments. Thus compound **1** was characterized as 7,4′-dihydroxy-2′-methoxy-6-(1″,1″-dimethylallyl)isoflav-3-ene, for which the trivial name burttinol-A is suggested.

Compound **2** is also an isoflav-3-ene derivative, having a hydroxyl, a methoxyl and a 2,2-dimethylpyran substituents. This was established from the UV, MS (M^+ at m/z 336), ^1H (Table 1) and ^{13}C (Table 2) NMR spectra. The base peak at m/z 321 $[M–Me]^+$ provided further support for the presence of a 2,2-dimethylpyran system. Comparison of the ^{13}C NMR spectral data of **2** with that of **1** (Table 2), revealed almost identical values for B-ring carbon atoms, indicating an identical substitution pattern in this ring. The placement of the methoxyl at C-2′ was established from the nOe interaction of the methoxy protons with the methylene protons at C-2 and H-3′. These nOe interactions were also observed in the mono-acetate (**2a**). Negative Gibb's reaction of **1** supports the placement of the methoxyl at C-2′.

The 2,2-dimethylpyran ring is then located in A-ring, between C-7 and C-8, the oxygen at C-7 (from biogenetic considerations). This is due to the presence of two *ortho*-coupled ($J=8.2$ Hz) aromatic protons at δ 6.81 (H-5) and 6.38 (H-6). The identity of this compound was proven through HMBC and HMQC experiments. Hence this new compound was identified as 4′-hydroxy-2′-methoxy-(2″,2″-dimethylpyrano[5″,6″:8,7]isoflav-3-ene (**2**) for which the trivial name, burttinol-B is suggested.

The third compound (**3**) is once again an isoflav-3-ene derivative having identical B-ring as in **1** and **2**. This was suggested from the ^1H (Table 1) and ^{13}C (Table 2) NMR spectra, which showed nearly identical data for this ring. The placement of the methoxyl at C-2′ was established from the nOe interaction of the methoxy protons with the methylene protons at C-2 and H-3′ as well as from negative Gibb's reaction. In the A-ring, the presence of a 3,3-dimethylallyl and a hydroxyl groups was evident from the MS, ^1H (Table 1) and ^{13}C (Table 2) NMR spectra. In the ^1H NMR spectrum, as in compound **2**, two *ortho*-coupled ($J=8.2$ Hz) aromatic protons at δ 6.82 (H-5) and 6.41 (H-6) were observed and require substituents at C-7 and C-8. Accordingly, the 3,3-dimethylallyl group is located at C-8 with the hydroxyl being at C-7, as expected from biogenetic considerations. It appears then that compound **3** is the precursor of **2**, whereby cyclization of the 3″, 3″-dimethylallyl group at C-8 and the hydroxyl group at C-7 produces the 2,2-dimethylpyran ring in **2**. Compound **3** was then characterized as 7,4′-dihydroxy-2′-methoxy-8-(3″,3″-dimethylallyl)isoflav-3-ene, for which the trivial name burttinol-C is suggested.

The UV (experimental), ^1H (δ 7.11, *s*, H-3) and ^{13}C (153.6 for C-2, 99.8 for C-3) NMR spectra of compound **4** were indicative of a 2-arylbenzofuran skeleton (Iinuma et al., 1994; Fukai et al., 1996). The presence of two hydroxyl, a methoxyl and a 1,1-dimethylallyl substituents were also evident from the MS ($[M]^+$ at m/z 324), ^1H and ^{13}C NMR spectra (see experimental). In the A-ring, the occurrence of two singlets at δ 7.43 (H-4) and 7.00 (H-7) would place the 1,1-dimethylallyl group at C-5 and a hydroxyl group at C-6. Oxygenation at C-6 is expected from biogenetic consideration, and the placement of the 1,1-dimethylallyl group at C-5 was confirmed from the NOE interaction of the methyl protons of this group with H-4.

In the B-ring, the occurrence of an AXY spin-system (δ 7.84, *d*, $J=9.1$ Hz, for H-6′; 6.52, *d*, $J=2.3$ Hz for H-3′ and 6.50, *dd*, $J=2.3$, 9.1 Hz, for H-5′) would require oxygenation at C-2′ and C-4′. The chemical shift values of the carbon atoms in this ring are similar with those of **1**, **2** and **3**, supporting such oxygenation pattern. The placement of the methoxyl at C-2′ was established from nOe interaction of the methyl protons with H-3. This compound also tested negative to Gibb's reaction, supporting the placement of the methoxyl at C-2′. Therefore, this new compound was characterized as 6,4′-dihydroxy-2′-methoxy-5-(1″,1″-dimethylallyl)-2-arylbenzofuran for which the trivial name burttinol-D is suggested.

The remaining compounds were identified as the flavanone, abyssinone V-4′-methyl ether (Yenesew et al., 1998b); the isoflav-3-ene, bidwillol A (Iinuma et al., 1994); the chalcone, isobavachalcone (Kobayashi et al., 1985; Yenesew et al., 1998b) and the pterocarpans, erythrabyssin II (Kamat et al., 1981; Mitscher et al., 1988), calopocarpin (Yenesew et al., 1998b), erybraedin A (Mitscher et al., 1988), phaseollidin (Kamat et al., 1981; Dagne et al., 1993) and phaseollin (Kamat et al., 1981; Telikepalli et al., 1990).

The root bark of *Erythrina burttii* mainly elaborate pterocarpans and isoflav-3-enes. Whereas several pterocarpans have been reported from the genus *Erythrina*, only one isoflav-3-ene, bidwillol A from *E. bidwillii*, (Iinuma et al., 1994), was known from this genus prior to this report.

3. Experimental

3.1. General

Analytical TLC: Merck pre-coated silica gel 60 F_{254} plates. CC on silica gel 60 (70–230 mesh). EIMS: direct inlet, 70 eV on a SSQ 710, Fa. Finnigan MAT spectrometer. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) on ARX 300 (Bruker) spectrometer using TMS as int. standard. HMQC and HMBC spectra were acquired using the standard Bruker software.

3.2. Plant material

The root bark of *Erythrina burttii* Ball. f. was collected near Emali town, on the Nairobi–Mombasa road, Kenya, in March 1995. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where a voucher specimen is deposited.

3.3. Extraction and isolation

Dried and ground root bark (190 g) of *Erythrina burttii* was extracted with acetone by cold percolation. Removal of the solvent afforded a brown gummy extract (10 g). The extract was subjected to flash CC on silica gel (100 g) eluting with petrol containing increasing percentages of EtOAc.

The fraction eluted with 3% EtOAc in petrol (800 ml) showed three spots on TLC and was separated by PTLC on silica gel plates (CH_2Cl_2, multiple development) to give erybraedin A (45 mg), phaseollin (23 mg) and abyssinone V-4′-methyl ether (38 mg). The fr. eluted with 5% EtOAc (800 ml) contained mainly two compounds, which were separated by PTLC on silica gel plates (CH_2Cl_2, multiple development) to give **2** (85 mg) and phaseollidin (26 mg). The fr. eluted with 10% EtOAc (600 ml) showed three spots on TLC and was separated by PTLC (CH_2Cl_2, multiple development) to give **4** (21 mg), bidwillol A (23 mg) and erythrabyssine II (29 mg). The fr. eluted with 15% EtOAc gave **1** (55 mg), while the components of the 20% EtOAc fraction (800 ml) were separated by PTLC (CH_2Cl_2/MeOH, 50:1; multiple development) to give **3** (18 mg) and calopocarpin (54 mg). Purification of the 30% EtOAc fraction (800 ml) by CC on sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) gave isobavachalcone (24 mg).

3.4. Burttinol A (*1*)

Oil, UV λ_{max} (MeOH) nm: 239, 328. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EIMS *m*/*z* (rel. int.): 338 (88, $[M]^+$), 323 (26), 215 (12), 161 (26), 147 (23), 141 (18), 137 (100). Compound **1** (15 mg) was treated with Ac_2O/pyridine at room temperature and worked up in the usual manner to give the diacetate **1a** (12 mg) as oil. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EIMS *m*/*z* (rel. int.): 422 (80, $[M]^+$), 379 (86), 337 (92), 137 (100).

3.5. Burttinol B (*2*)

Oil, UV λ_{max} (MeOH) nm: 244, 320. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EIMS *m*/*z* (rel. int.): 336 (63, $[M]^+$), 321 (100), 197 (18), 165 (18), 161 (63), 147 (19), 141 (19), 137 (25). Compound **2** (10 mg) was acetylated as above to give a monoacetate **2a** (7 mg) as oil. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EIMS *m*/*z* (rel. int.): 378 (55, $[M]^+$), 363 (100), 335 (67), 321 (94).

3.6. Burttinol C (*3*)

Oil, UV λ_{max} (MeOH) nm: 241, 325. ^{1}H NMR (Table 1). ^{13}C NMR (Table 2). EIMS *m*/*z* (rel. int.): 338 (100, $[M]^+$), 323 (26), 282 (98, $[M–C_4H_8]^+$), 161 (27), 147 (24), 141 (19), 137 (32).

3.7. Burttinol D (*4*)

Oil, UV λ_{max} (MeOH) nm: 231, 285, 326 341. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.11 (1H, *s*, H-3), 7.43 (1H, *s*, H-4), 7.00 (1H, *s*, H-7), 6.52 (1H, *d*, $J = 2.3$ Hz, H-3′), 6.50 (1H, *dd*, $J = 2.3$, 9.1 Hz, H-5′),), 7.84 (1H, *d*, $J = 9.1$ Hz, H-6′),), 6.24 (1H, *dd*, $J = 10.5$, 17.7 Hz, H-2″), 5.29 (1H, *d*, $J = 10.5$ Hz, H-3″), 5.36 (1H, *d*, $J = 17.7$ Hz, H-3″), 1.50 (6H, *s*, 1″-Me_2), 3.94 (3H, *s*, OMe). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.6 (C-2), 99.8 (C-3), 112.9 (C-3a), 117.4 (C-4), 128.4 (C-5), 152.4 (C-6), 104.1 (C-7), 151.6 (C-7a), 123.4 (C-1′), 157.5 (C-2′), 99.2 (C-3′), 156.6 (C-4′), 107.4 (C-5′), 127.7 (C-6′), 40.4 (C-1″), 148.1 (C-2″), 113.7 (C-3″), 27.4 (1″-Me_2). EIMS *m*/*z* (rel. int.): 324 (54, $[M]^+$), 281 (11), 159 (31).

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