Phytochemistry 70 (2009) 216–221

Contents lists available at ScienceDirect

Phytochemistry

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Prenylated arylbenzofuran derivatives from *Morus mesozygia* with antioxidant activity

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ARTICLE INFO

Article history:
Received 16 September 2008
Received in revised form 4 December 2008
Available online 13 January 2009

Keywords:
Artocarpesine
Morachalcone A
Moracin
Marsformoxide
Morus mesozygia
Moraceae
Prenylated arylbenzofurans
Antioxidant activity

ABSTRACT

Five prenylated arylbenzofurans, moracins Q–U, were isolated from *Morus mesozygia* (Moraceae). Their structures were elucidated on the basis of spectroscopic evidence. Along with these compounds, 3β-acetoxyurs-12-en-11-one, marsformoxide, moracin C, moracin M, moracin K, artocarpesin, cycloartocarpesin, morachalcone A were also isolated. Four of the five compounds, (moracins R–U) displayed potent antioxidant activity.

1. Introduction

In the course of our systematic phytochemical and pharmacological studies of the Cameroonian Moraceous plants (Ngadjui et al., 1999a,b, 2000; Kapche et al., 2007; Metuno et al., 2008) we have investigated *Morus mesozygia* Stapf. *Morus* or Mulberry is a genus of 10–16 species of deciduous trees native to warm, temperate, and subtropical regions of Asia, Africa, and the Americas, with the majority of the species native to Asia (9 in China) (Venkatesh and Seema, 2008). Leaves of *Morus* species, especially, of *M. alba* have been an indispensable food source for silk-worms, and the root barks of *M. macroura* have been used to treat diabetes, arthritis and rheumatism in Chinese herbal medicine (Sheng-Jun et al., 2004). *M. mesozygia* (black mulberry) is a small to medium sized forest tree of Tropical Africa. Its leaves and fruit provide food for the *Mantled Guereza*, a colobus monkey native to much of Tropical Africa, and for the common chimpanzee of West and Central Africa (Fashing, 2001). *M. mesozygia* is used to cure arthritis, rheumatism, malnutrition, debility, stomach troubles, venereal diseases and as pain killer (Burkill, 1985). *Morus* species have been the subject of many phytochemical and pharmacological studies and a number of pharmacologically active compounds have been isolated (Nomura et al., 1982, 1983; Takasugi et al., 1982; Hano et al., 1984, 1988a,b; Hirakura et al., 1985a,b; Matsuyama et al., 1991a; Basnet et al., 1993; Syah et al., 2000; Shen and Lin, 2001; Sang-Hee et al., 2002; Sheng-Jun et al., 2004; Young-Woong et al., 2005). These biological properties, together with the fact that *M. mesozygia* is a plant that is hitherto not so well studied, prompted us to undertake a phytochemical investigation of this plant. Thirteen compounds (**1**–**13**) were isolated from the MeOH extract, of which, moracin R (**2**), moracin S (**3**), moracin T (**4**) and moracin U (**5**) exhibited potent antioxidant activities.

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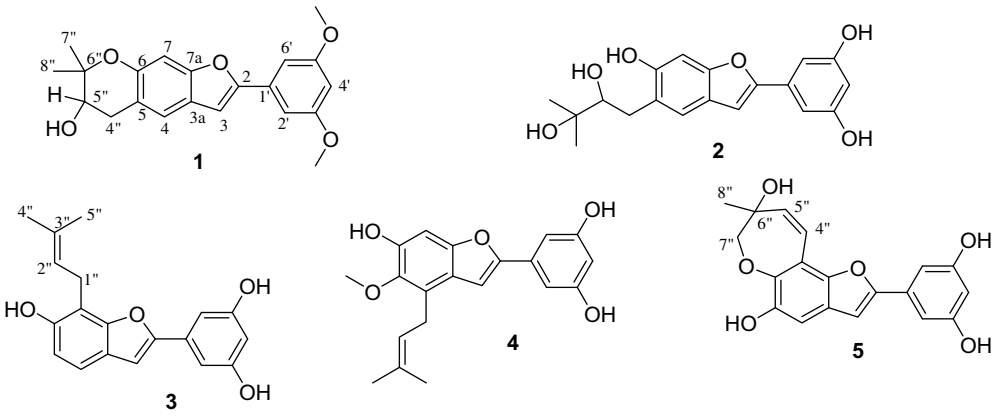
doi:10.1016/j.phytochem.2008.12.014



2. Results and discussion

The MeOH extract of the trunk bark of *M. mesozygia* was subjected to column chromatography over silica gel, and Sephadex LH-20 to give thirteen compounds, including five new stilbenoids moracins Q–U. The present paper deals with the isolation and structure elucidation of these compounds.

Compounds **1**–**5** were characterized as 2-arylbenzofuran derivatives and were noted to have the following common features. Their UV spectra displayed the characteristic two absorption bands in the regions 204–237 and 290–320 nm (Nomura and Fukai, 1981; Pacher et al., 2002). Compounds **2**–**5** gave the expected colours upon reaction with methanolic ferric chloride confirming the presence of free phenolic hydroxyl substituents. The IR spectra also showed absorptions at ca. 3400 (–OH stretch) and the typical aryl absorptions and overtones from 1610–1450 cm^{-1}.

Moracin Q (**1**) was obtained as a pink amorphous powder. The molecular formula was determined to be $C_{21}H_{22}O_5$ by HREI-MS, which showed the molecular ion peak at *m*/*z* 354.1463 (calc. 354.1467) and NMR. The ^{1}H NMR spectrum of compound **1** disclosed the presence of ring A of a disubstituted 2-arylbenzofuran moiety 7.19 (1H, *s*, H-3), δ 7.37 (1H, *s*, H-4), 6.89 (1H, *s*, H-7); a trisubstituted 2,2-dimethyldihydropyran ring at [δ 1.25 (3H, *s*), 1.29 (3H, *s*), 3.22 (1H, *dd*, *J* = 9.0, 15.6 Hz, H-4b″), 3.33 (1H, *dd*, *J* = 8.4 and 15.6 Hz, H-4a″), 4.71 (1H, *t*, *J* = 8.7 Hz, H-5″)]. The remaining signals at 7.02 (2H, *d*, *J* = 2.1 Hz, H-2′, H-6′), 6.49 (1H, *t*, *J* = 2.1 Hz, H-4′) were assigned to the trisubstituted ring-B. The ^{13}C NMR spectrum indicated 21 carbons, including two methyl groups, two methoxyl, one methylene, one sp^3 oxymethyne, six sp^2 methyne and four oxyaryl carbons. The location of the dihydropyran ring and the methoxyl groups (δ 3.87 (6H, *s*) on the 2-arylbenzofuran moiety were determined by HMBC (Fig. 1). Therefore, moracin Q was assigned the name [2″,3″: 5,6]-(5-hydroxy-4,5-dihydro-6,6-dimethylpyrano)-2-(3,5-dimethoxyphenyl)benzofuran. The demethylated derivative, moracin P, has been reported from *M. alba* (Hirakura et al., 1996).

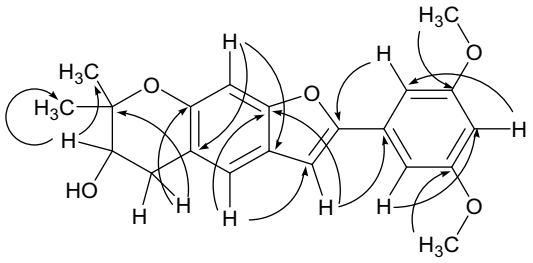


Fig. 1. Key HMBC correlations of **1**.

Moracin R (**2**) was obtained as brown oil, and gave a dark blue colour with methanolic ferric chloride. The molecular formula was determined to be $C_{19}H_{20}O_5$ by HREI-MS (calc.: 328.1311) and NMR. The ^{1}H NMR spectrum of compound **2** was similar to that of **1** in showing signals consistent with the presence of a disubstituted 2-arylbenzofuran moiety and also similar substitution pattern in ring-B. However, this compound did not show any methoxyl resonances. From this observation and the molecular formula $C_{19}H_{20}O_5$ it was possible to conclude that moracin R contained one less degree of unsaturation and it was assumed that the substitution at C-5 is a non-cyclized dihydroxy prenyl group. This assumption was supported by the proton spectrum which showed the presence of two methyl groups at δ 1.28 (3H, *s*) and 1.38 (3H, *s*), one oxymethyne proton at δ 3.84 (1H, *dd*, *J* = 5.4 and 7.9 Hz), and two methylene protons at δ 3.12 (1H, *dd*, *J* = 5.4 and 17.0 Hz), 2.83 (1H, *dd*, *J* = 7.8 and 17.0 Hz). The ^{13}C NMR spectrum of **2** showed signals of the oxymethyne carbon at δ 70.0, the methylene carbon at δ 32.4, and a quaternary carbon at δ 78.1. The HMBC experiments showed correlations between these carbons and the above protons indicating the presence of a 2,3-dihydroxy-3-methylbutyl substituent. The methylene protons at δ 2.83 and 3.12 (H-1″) also showed correlations with one quaternary aryl carbons at δ 117.9 (C-5), one oxyaryl carbon at δ 152.5 (C-6) and one aryl-methyne carbon at 121.7 (C-4). The aromatic protons at δ 6.90 (H-7) and δ 7.25 (H-4) showed HMBC with four quaternary carbons: at δ 117.9 (C-5), 123.5 (C-3a), 152.5 (C-6) and 155.4 (C-7a). There was also a ^{3}J correlation between the aromatic proton at δ 7.25 (H-4) and the methylene carbon at δ 32.4 (C-1″). These results confirmed the presence of the 2,3-dihydroxy-3-methylbutyl substituent at C-5. Consequently, moracin R was assigned the name 5-(2,3-dihydroxy-3-methylbutyl)-2-(3,5-dihydroxyphenyl)benzofuran-6-ol.

Moracin S (**3**) was obtained as a brown amorphous powder, and gave a dark violet colour with methanolic ferric chloride. The molecular formula was determined to be $C_{19}H_{18}O_4$ by HREI-MS which showed the molecular ion peak at *m*/*z* 310.1199 (calc. 310.1205) and NMR. Like compound **2**, this compound also showed the general features described in the beginning of the Section. Its ^{1}H NMR spectrum disclosed the presence of a disubstituted 2-arylbenzofuran moiety [δ 7.24 (1H, *d*, *J* = 8.4 Hz, H-4), 7.05 (1H, *s*, H-3), 6.85 (1H, *d*, *J* = 8.4 Hz, H-5), 6.92 (2H, *d*, *J* = 2.1 Hz, H-2′, H-6′), 6.39 (1H, *t*, *J* = 2.1 Hz, H-4′) and nine protons attributable to a prenyl group at δ 5.05 (1H, *m*, H-2″), 3.66 (2H, *d*, *J* = 7.2 Hz, H-1″), 1.90 (3H, *s*, H-4″) and 1.69 (3H, *s*, H-5″). The ^{13}C NMR spectrum indicated 19 carbons, including two methyl groups, one methylene, seven sp^2 methyne and four oxyaryl carbons. The location of the prenyl group on the 2-arylbenzofuran moiety was determined by HMBC experiment, and key HMBC are listed in Table 1. Therefore, the structure of Moracin S was determined as: 2-(3,5-dihydroxyphenyl)-7-(3-methylbut-2-enyl)benzofuran-6-ol. Moracin S may

Table 1
^{1}H (300 and 600 MHz, 25 °C) and ^{13}C NMR (75 and 150 MHz, 25 °C) spectral data of **1**, **2**, **3**, **4** and **5** in acetone-d_6.

Position	**1**		
	^{1}H, *m*, *J*(Hz)	^{13}C	HMBC (H → C)
1	–	–	
2	–	155.6	
3	7.19, *s*	103.6	117.5, 123.7, 133.9, 155.6, 156.4
3a	–	123.7	
4	7.37, *s*	117.5	31.1, 103.6, 123.7, 125.9, 156.4, 160.3
5	–	125.9	
6	–	160.3	
7	6.89, *s*	93.5	123.7, 125.9, 156.4, 160.3
7a	–	156.4	
1′	–	133.9	
2′	7.02, *d*, 1.8	103.4	101.4, 133.9, 155.6, 162.7
3′	–	162.7	
4′	6.49, *t*, 2.1	101.4	103.4, 162.4
5′	–	162.7	
6′	7.02, *d*, 1.8	103.4	101.4, 133.9, 155.6, 162.7
4″a	3.33, *dd*, 8.4;15.6	31.1	71.9, 91.3, 117.5, 125.9, 160.3
4″b	3.22, *dd*, 9.0;15.6		
5″	4.71, *t*; 8.7	91.7	26.0, 26.5, 31.1, 125.9
6″		71.9	
7″a	1.25, *s*	26.5	26.0, 71.9, 91.3
7″b			
8″	1.29, *s*	26.0	26.5, 71.9, 91.3
3′–OCH_3	3.87, *s*	56.2	162.7
5′–OCH_3	3.87, *s*	56.2	162.7
Position	**2**		
	^{1}H, *m*, *J*(Hz)	^{13}C	HMBC (H → C)
1	–	–	
2	–	155.5	
3	7.00, *s*	101.3	123.5, 133.3, 155.4, 155.5
3a	–	123.5	
4	7.25, *s*	121.7	32.4, 123.5, 152.5, 155.4
5	–	117.9	
6	–	152.5	
7	6.90, *s*	94.4	117.9, 123.5, 152.5,
7a	–	155.4	
1′	–	133.9	
2′	6.88, *d*, 2.1	103.9	103.6, 155.5, 159.8
3′		159.8	
4′	6.39, *t*, 2.1	103.6	103.9, 159.8
5′	–	159.8	
6′	6.88, *d*, 2.1	103.9	103.6, 155.5, 159.8
1″a	3.12, *dd*, 5.4;16.5	32.4	70.0, 78.1, 117.9, 121.7, 152.5
1″b	2.83, *dd*, 8.1;17.4		
2″	3.84, *dd*, 5.4;7.8	70.0	20.7, 26.2, 78.1, 117.9
3″	–	78.1	
4″	1.28, *s*	20.7	26.2, 70.0, 78.1
5″	1.38, *s*	26.2	20.7, 70.0, 78.1
6″	–	–	
5–OCH_3	–	–	
Position	**3**		
	^{1}H, *m*, *J*(Hz)	^{13}C	HMBC (H → C)
1	–	–	
2	–	155.6	
3	7.05, *s*	102.8	122.7, 133.8, 153.7, 155.6
3a	–	122.7	
4	7, 24, *d*, 8.4	119.1	102.8, 153.7, 155.5
5	6.85, *d*, 8.4	113.3	112.3, 122.7
6	–	155.5	
7	–	112.3	
7a	–	153.7	
1′	–	133.8	
2′	6, 92, *d*, 2.1	104.0	103.6, 155.6, 160.0
3′		160.0	
4′	6.39, *t*, 2.1	103.6	104.0, 160.0
5′	–	160.0	
6′	6, 92, *d*, 2.1	104.0	103.6, 155.6, 160.0
1″a	3.66, *d*, 7.2	23.0	112.3, 123.3, 132.1, 153.7, 155.5
1″b			
2″	5.46, m	123.3	
3″	–	132.1	
4″	1.69, *s*	26.1	18.2, 123.3, 132.1
5″	1.90, *s*	18.2	26.1, 123.3, 132.1
6″	–	–	–
5–OCH_3	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Position	**1**		
Position	**4**		
	^{1}H, *m*, *J*(Hz)	^{13}C	HMBC (H → C)
1	–	–	
2	–	155.6	
3	7.05, *s*	101.9	123.3, 152.5, 155.6
3a	–	122.3	
4	–	127.6	
5	–	144.0	
6	–	150.0	
7	6.93, *s*	97.4	122.3, 144.0, 150.0, 152.5
7a	–	152.5	
1′	–	133.7	
2′	6.86, *d*, 2.1	104.0	103.6, 155.6, 160.0
3′	–	160.0	
4′	6.37, *t*, 2.1	103.6	104.0, 160.0
5′	–	160.0	
6′	6.86, *d*, 2.1	104.0	103.6, 155.6, 160.0
1″a	3.59, *d*, 6.9	27.3	122.3, 124.1, 127.6, 132.5, 144.0
1″b			
2″	5.32, *m*	124.1	–
3″		132.5	
4″	1.71, *s*	26.1	18.3, 124.1, 132.5
5″	1.86, *s*	18.3	26.1, 124.1, 132.5
6″	–	–	
5–OCH$_3$	3.79, *s*	61.8	144.0
Position	**5**		
	^{1}H, *m*, *J*(Hz)	^{13}C	HMBC (H → C)
1	–	–	
2	–	156.6	
3	7.04, *s*	102.8	124.2, 147.9, 156.6
3a	–	124.2	
4	7.00, *s*	105.7	102.8, 146.0, 145.7, 147.9
5	–	146.0	
6	–	145.7	
7	–	112.2	
7a	–	147.9	
1′	–	133.5	
2′	6.91, *d*, 1.8	104.2	104.0, 156.6, 160.2,
3′		160.2	
4′	6.40, *ps*	104.0	104.2, 160.2
5′		160.2	
6′	6.91, *d*, 1.8	104.2	104.0, 156.6, 160.2
4″a	6.89, *d*, 12.3	117.4	112.2, 140.1, 145.7, 147.9
4″b			
5″	6.16, *d*, 12.3	140.1	112.2, 117.4
6″		73.3	
7″a	4.10, *d*, 11.4	79.9	26.6, 73.3, 140.1, 145.7
7″b	4.15, *d*, 11,4		
8″	1.40, *s*	26.6	73.3, 79.9, 140.1
3′–OCH$_3$	–	–	
5′–OCH$_3$			

be referred to as 7-prenylmoracin M, but unfortunately this name has been incorrectly assigned to the 5-hydroxy, $\delta^{1'',2''}$ isomer which was isolated recently from *Artocarpus dadah* (Su et al., 2002).

Moracin T (**4**) was obtained as yellow oil, and gave a dark green colour with the methanolic ferric chloride. The molecular formula was determined to be $C_{20}H_{20}O_5$ by HREI-MS which showed the molecular ion peak at *m*/*z* 340.1306 (calc. 340.1311) and NMR. The ^{1}HNMR spectrum of compound **4** showed the presence of a trisubstituted 2-arylbenzofuran moiety [δ 7.05 (1H, *s*, H-3), 6.93 (1H, *s*, H-7), 6.88 (2H, *d*, *J* = 2.1 Hz, H-2′, H-6′), 6.39 (1H, *t*, *J* = 2.1 Hz, H-4′). The ^{1}H NMR data also indicated the presence of two methyl groups at δ 1.71 (3H, *s*) and 1.86 (3H, *s*), one olefinic proton at δ 5.32 (1H, *m*), and a pair of methylene protons at δ 3.59 (2H, *d*, *J* = 6.9 Hz), attributed to a 3-methyl-2-butenyl (isoprenyl) substituent. In addition, the ^{1}H NMR spectrum also indicated the presence of a methoxyl groups at δ 3.79 (3H, *s*). The ^{13}C NMR spectrum indicated 20 carbons, including two methyl groups, one methoxyl, one methylene, six sp^2 methyne and five oxyaryl carbons. The HMBC measurements showed long-range correlations between the methylene protons at δ 3.59 (H-1″) and four quaternary carbons at δ 122.3 (C-3a), 127.6 (C-4), 132.5 (C-3″) and 144.0 (C-5). The HMBC experiments also showed the connectivities (Table 1) between the methoxyl protons at δ 3.79 and the quaternary carbon at δ 144.0 (C-5), also between the aromatic proton at δ 6.93 (C-7) and 4 quaternary carbons at δ 122.3 (C-3a), 144.0 (C-5), 150.0 (C-6) and 152.5 (C-7a). These results provided support for the presence of the prenyl substituent at C-4, and the methoxyl group at C-5. Thus moracin T was finally assigned the name 2-(3,5-dihydroxyphenyl)-5-methoxy-4-(3-methylbut-2-enyl)benzofuran-6-ol.

Moracin U (**5**) was obtained as yellow oil, and gave a dark green colour with methanolic ferric chloride. The molecular formula was determined to be $C_{19}H_{16}O_6$ by HREI-MS which showed the molecular ion peak at *m*/*z* 340.0945 (calc. 340.0947) and NMR. The ^{1}H NMR spectrum of compound **5** disclosed the presence of a trisubstituted 2-arylbenzofuran moiety [δ 7.04 (1H, *s*, H-3), 7.00 (1H, *s*, H-4), 6.91 (2H, *d*, *J* = 2.1 Hz, H-2′, H-6′), 6.40 (1H, *t*, *J* = 2.1 Hz, H-4′), a tetrasubstituted oxacycloheptene ring at [δ 6.89 (1H, *d*, *J* = 12.3, H-4″), 6.16 (1H, *d*, *J* = 12.3, H-5″), 4.15(1H, *d*, *J* = 11.4,

Table 2
Antioxidant activities of the crude extract and the new isolated compounds.

Compounds	Concentrations of tested compounds and percentages of free radical scavenging activities				EC50 (μg/ml)
	12.5 (μg/ml)	25 (μg/ml)	50 (μg/ml)	100 (μg/ml)	
Trolox (VitE)	70.79 ± 01.98	76.72 ± 03,29	80.50 ± 02.77	93.39 ± 01.98	03.47 ± 01.55
4	61.58 ± 02.78	65.63 ± 02.33	71.72 ± 04.65	83.04 ± 01.84	04.12 ± 02.73
3	55.00 ± 01.76	62.25 ± 00.75	67.87 ± 01.65	69.72 ± 00.83	05.06 ± 01.41
Crude extract	38.10 ± 00.99	44.82 ± 03.76	47.98 ± 2.88	54.88 ± 01.65	05.92 ± 01.09
5	38.50 ± 03.65	42.09 ± 01.76	49.98 ± 02.66	53.60 ± 01.90	06.08 ± 02.32
2	34.60 ± 02.76	38.20 ± 01.45	48.00 ± 02.54	50.00 ± 03.32	07.17 ± 01.98

Values are percentage of discoloration and EC_{50} ± SD of two experiments in triplicate. Trolox: antioxidant reference compound (hydrosoluble form of Vit. E).

H-7a″), 4.10 (1H, *d*, *J* = 11.4, H-7b″)], one methyl group at δ 1.40 (3H, *s*) and three hydroxyl groups δ 8.56 (2H, *s*), 7.64 (1H, *s*) and 4.35 (1H, *s*). The ^{13}C NMR spectrum indicated 19 carbons, including one methyl group, one methylene carbon, six sp^2 methyne carbons, and five oxyaryl carbons. The HMBC between the methyl protons at δ 1.40 and the methylene carbon at δ 79.9 (C-7″), the quaternary carbon at δ 73.0 (C-6″) and the sp^2 methyne carbon at δ 140.1 (C-5″) as well as the correlation between the hydroxyl group at δ 4.35 and the quaternary carbon at 73.0 (C-6″) allowed us to locate these two groups at C-6″ on the oxacycloheptene ring. The location of the oxacycloheptene ring, and the other hydroxyl groups on the 2-arylbenzofuran moiety was determined by HMBC measurements, and key HMBC are shown in Table 1. Therefore, moracin U was finally assigned the structure of [2″,3″:6,7]-(6,7-dihydro-6-hydroxy-6-methyloxepine)-2-(3,5-dihydroxyphenyl)benzofuran-5-ol. Such unusual prenyl cyclisation to an oxacycloheptene ring has been noted in moracin L, isolated from diseased mulberry (Matsuyama et al., 1991a,b).

The known compounds α-amyrinone acetate **6**, marsformoxide **7**, moracin C **8**, moracin M **9**, moracin K **10**, artocarpesine **11**, cycloartocarpesine **12**, morachalcone A **13** were identified by comparison of their spectral data (^1H NMR, ^{13}C NMR, MS) with reported values.

The free radical-scavenging activities of the crude extract and compounds **1–5** was evaluated by assessing their ability to decolorize DPPH (2,4-dinitrophenyl-1-picrylhydrazyl) in methanol (BrandWilliams et al., 1995) and percentages of discoloration and concentration of the sample required to scavenge 50% DPPH (EC_{50}) are shown in Table 2. Based on the bioassay results, it is concluded that compound **4** possess antioxidant activity close to that of the positive control (Vit E). No antioxidant activity was shown with compound **1**.

Benzofurans and some phenolic compounds are widely distributed in the Moraceae family in general and in *Morus* in particular and are known to exhibit strong antifungal activities, especially, from mulberry trees infected with *Fusarium solani*. Benzofurans may be tentatively considered as marker of the *Morus* genus. Therefore, the isolation of the new arylbenzoforans from the sole African species of the genus is not surprising. Moracin Q (**1**) is the demethylated derivative of moracin P which was isolated from M. alba for the first time (Hirakura et al., 1996). Moracin R (**2**) can be considered as the 2,3-dihydroxyprenyl derivative of moracin N which was reported from *M. alba* (Matsuyama et al., 1991b); moracin S (**3**) is an isomer of moracin N (5-prenylmoracin M), where the prenyl group is attach at position **7**, instead of position **5**. Moracin T (**4**) can be considered as the demethylated and prenylated derivative of moracin F isolated from diseased mulberry (Takasugi et al., 1979), whereas moracin U (**5**) is probably the precursor of moracin L.

The results of this investigation of *M. mesozygia* indicated that this plant, like the other species of the genus, is a rich source of arylbenzofurans. Moreover this study identifies *M. mesozygia* as a rich source of moracin M which can be considered here as the biogenetic precursor of the arylbenzofurans isolated from this plant.

3. Experimental

3.1. General

UV spectra were obtained on a Shimadzu UV-210 IPC UV–vis scanning spectrophotometer. NMR spectra were recorded on a Bruker Avance 300 at 300 MHz (^1H) and 75 MHz and Bruker Avance 600 at 600 MHz (^1H) and 150 MHz (^{13}C), with the residual solvent peaks as internal references. IR spectra were recorded on a Shimadzu FTIR-8700 Fourier Transform Infrared spectrometer with KBr disks. Optical rotations were measured on a Polartronic D eloptron Schimdt + Haensch polarimeter and Autopol IV automatic polarimeter Rudolph research analytical. MS were obtained with a VG Autospec mass spectrometer, using the EI mode. Silica gel (200–300 mesh) for CC. Precoated plates of silica gel GF254 were used for TLC, and detected under UV light and sprayed with concentrated sulphuric acid. All reagents used for the antioxidant activity testing were of high purity and purchased from SIGMA Chemicals Co. (Dorset, UK) and Prolabo (Paris, France).

3.2. Plant material

The trunk bark of *M. mesozygia* was collected in Yaoundé, in the Centre Province, Cameroon, in June 2007, and identified at the National Herbarium were a voucher specimen N° 4228/SRFK is deposited.

3.3. Extraction and isolation

The air-dried trunk barks (2 kg) of *M. mesozygia* were powdered and extracted with MeOH at room temperature for 48 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (25 g), which was subjected to CC on silica gel (200–300 mesh), eluted with hexane and ethyl acetate in increasing polarity, and the fractions were combined according to TLC monitoring to give eight fractions. Fraction I (hexane–ethyl acetate 9/2, 2.5 g) was re-subjected to CC on silica gel (200–300 mesh) [eluted with hexane–ethyl acetate (95/5, v/v)] giving 3β-acetoxyurs-12-en-11-one **6** (20 mg), marsformoxide **7** (30 mg). Fraction IV (hexane–ethyl acetate 7.5/1.5, 0.7 g) was subjected to CC over Sephadex LH-20 [$CHCl_3$–MeOH, 7/3, v/v] and on silica gel (200–300 mesh) [eluted with $CHCl_3$–MeOH (97/3, v/v)] to give **1** (15 mg). Fraction V (hexane–ethyl acetate 6/4, 2.0 g) was subjected to CC on silica gel (200–300 mesh) [eluted by $CHCl_3$–MeOH, 95/5–90/10–85/15, v/v] giving **4** (60 mg), **10** (100 mg), **11** (75 mg), **12** (45 mg). Fraction VI (hexane–ethyl acetate 1/1–2/8, 16.0 g) was subjected to successive CC on silica gel (200–300 mesh) [eluted by $CHCl_3$–MeOH, 95/5–90/10–85/15, v/v], Sephadex LH-20 [$CHCl_3$–MeOH, 7/3 and ethyl

acetate–MeOH 9/1 v/v] to give **4**, **10**, **11**, **12**, **2** (300 mg), **3** (37 mg), **5** (50 mg), **8** (54 mg), **9** (1 g) and **13** (10 mg).

3.3.1. Moracin Q (**1**)

Pink amorphous powder; $[\alpha]_D^{20}$+17 (*c* 0.13, MeOH); UV: λ_{max}^{MeCOMe} nm: 213, 282, 310; IR: ν_{max}^{KBr} cm^{-1}: 3433, 1608, 1454, 1157; ^1H NMR (600 MHz, CD_3COCD_3) and ^{13}C NMR (150 MHz, CD_3COCD_3) data see Table 1; HREI-MS *m*/*z*: 354.1463 (calc. for $C_{21}H_{22}O_5$).

3.3.2. Moracin R (**2**)

Brown oil; $[\alpha]_D^{20}$ −25 (*c* 0.10, MeOH); UV: λ_{max}^{MeCOMe} nm: 216, 261, 282, 299, 315; IR: ν_{max}^{KBr} cm^{-1}: 3394, 1600, 1578, 1462, 1354, 1146; ^1H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (75 MHz, CD_3COCD_3) data see Table 1; HREI-MS *m*/*z*: 326.1147 (calc. for $C_{19}H_{18}O_5$ [M-H_20]$^{+\cdot}$); 255.0643 (calc. for $C_{15}H_{11}O_4$ [M-$C_4H_{10}0_4$]$^{+\cdot}$).

3.3.3. Moracin S (**3**)

Brown amorphous powder: UV: λ_{max}^{MeCOMe} nm: 219, 244, 255, 255, 303, 310; IR: ν_{max}^{KBr} cm^{-1}: 3417, 1697, 1620, 1578, 1423, 1157; ^1H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (75 MHz, CD_3COCD_3) data see Table 1; HREI-MS *m*/*z*: 310.1199 (calc. for $C_{19}H_{18}O_4$); 255.0644 (calc. for $C_{15}H_{11}O_4$ [M-C_4H_7]$^{+\cdot}$).

3.3.4. Moracin T (**4**)

Yellow oil; UV: λ_{max}^{MeCOMe} nm: 224, 240, 267, 279, 299; IR: ν_{max}^{KBr} cm^{-1}: 3421, 1635, 1543, 1508, 1458, 1153; ^1H NMR (300 MHz, CD_3COCD_3) and ^{13}C NMR (75 MHz, CD_3COCD_3) data see Table 1; HREI-MS *m*/*z*: 340.1306 (calc. for $C_{20}H_{20}O_5$).

3.3.5. Moracin U (**5**)

Colorless amorphous powder; $[\alpha]_D^{20}$+19 (*c* 0.50, MeOH); UV: λ_{max}^{MeCOMe} nm: 211, 288, 311, 336; IR: ν_{max}^{KBr} cm^{-1}: 3421, 1620, 1542, 1458, 1153; ^1H NMR (600 MHz, CD_3COCD_3) and ^{13}C NMR (150 MHz, CD_3COCD_3) data see Table 1; HREI-MS *m*/*z*: 340.0945 (calc. for $C_{19}H_{16}O_6$).

3.4. Antioxidant activity

The free radical-scavenging activity of the compounds was evaluated by assessing their ability to discolour –DPPH (2,4-dinitrophenyl-1-picrylhydrazyl) in methanol according to BrandWilliams et al. (1995). Each compound was tested at concentrations of 12.5; 25; 50 and 100 μg/ml. The decrease in absorbance was monitored at 517 nm and exactly 30 s after adding the appropriate volume of the extract or methanol to the blank. Then the percentage of discoloration was calculated for the determination of the concentration of the sample required to scavenge 50% DPPH (EC_{50}) which were estimated using Graph Pad Prism 3.0.

Acknowlegements

We thank Mr. V. Ndlovu, Senior Technician of the University of Botswana, for UV, IR and optical rotation measurements; the Network for Analytical and Bio-assay Services in Africa (NABSA) for the financial support; and the African Network of Scientific and Technological Institutions (ANSTI) for a three month travel grant to the Department of Chemistry, University of Botswana.

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