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Diarylheptanoids from *Myrica arborea*

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Abstract

Investigations of the stem and root bark of *Myrica arborea* (Myricaceae) have yielded two novel diarylheptanoids, myricarborin and 11-*O*-β-D-xylopyranosylmyricanol along with the known myricanol and 5-*O*-β-D-glucopyranosylmyricanol. The structures of the novel compounds were determined by spectroscopic methods.

Keywords: *Myrica arborea*; Myricaceae; Diarylheptanoids; Myricarborin; 11-*O*-β-D-xylopyranosylmyricanol; 5-*O*-β-D-glucopyranosylmyricanol

1. Introduction

Myrica arborea Hutch (Myricaceae) is a montane tree of the Western highlands of Cameroon (Hutchinson and Dalziel, 1958). Its bark decoctions are a reputed remedy for fevers and inflammations among the Bamilike tribesmen of Cameroon. Diarylheptanoids and triterpenes have been documented previously as constituents of plants classified in the genus *Myrica* (Begley et al., 1971; Joshi et al., 1996; Malterud et al., 1976; Sakurai et al., 1991; Sun et al., 1988; Yaguchi et al., 1988). However, no work has been reported on *M. arborea*. As part of our continuing studies on plants used against fevers (Tchuendem et al., 1999), we have isolated four cyclic diarylheptanoids from the bark of *M. arborea*. This paper describes the isolation and structural elucidation of two novel natural products, myricarborin (**1**) and 11-*O*-β-D-xylopyranosylmyricanol (**2**), as well as the identification of the known myricanol (**3**) (Joshi et al., 1996) and 5-*O*-β-D-glucopyranosylmyricanol (**4**) (Inoue et al., 1984).

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2. Results and discussion

The MeOH–CH_2Cl_2 (1:1) extract of *M. arborea* was subjected to a sequential liquid–liquid partition with hexanes, CH_2Cl_2, and EtOAc. The combined CH_2Cl_2 and EtOAc fractions were fractionated by Si gel column chromatography to give several fractions which were further purified by Sephadex LH-20 permeation and medium pressure liquid chromatography to afford the diarylheptanoids **1**, **2**, **3**, and **4** (Fig. 1).

Compounds **3** and **4** were identified respectively as myricanol (Joshi et al., 1996) and 5-*O*-β-D-glucopyranosylmyricanol (Inoue et al., 1984) by comparison of their melting points, IR spectral data, and $[\alpha]_D$ with those reported in the literature. Compound **1**, to which we have given the trivial name myricarborin, and compound **2** (11-*O*-β-D-xylopyranosylmyricanol) are novel.

Myricarborin (**1**) was obtained as colourless plates from CH_2Cl_2, mp 141–143°, $[\alpha]_D + 9.3°$. The EIMS showed a molecular ion peak at m/z 310, and the CIMS pseudomolecular ion peaks at m/z 311 $[M + H]^+$, 325 $[M + CH_3]^+$, and 339 $[M + C_2H_5]^+$. These data were in agreement with the molecular formula $C_{20}H_{22}O_3$. This molecular formula was confirmed by the HREIMS which showed the molecular ion peak at

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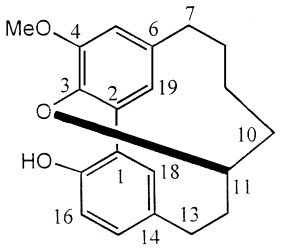
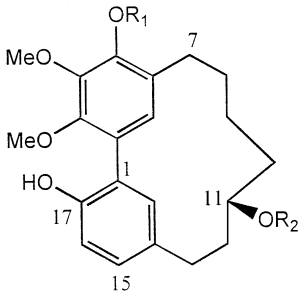
m/z 310.1583 ($C_{20}H_{22}O_3$ requires: 310.1569). The IR spectrum showed strong absorptions at ν_{max} 3420, 1620, and 1601 cm^{-1} indicative of a hydroxylated aromatic ring system. Analysis of the ^1H- and ^{13}C-NMR spectra of **1** indicated that it was a cyclic diarylheptanoid closely related to myricanol (**3**) but differing in the absence of oxygenation at the C-5 position. In the ^1H NMR spectrum of **1**, five aromatic proton signals were observed as two broad singlets of one proton each at δ 6.51 and 6.70, and as an ABX system at δ 6.65 (d, $J = 8.1$ Hz), 6.87 (dd, $J = 2.1, 8.1$ Hz), and 7.04 (d, $J = 2.1$). A conspicuous signal for a methoxyl group was also observed at δ 3.77. These six resonances were assigned to H-5, H-19, H-16, H-15, H-18, and CH_3O-4, respectively. Apart from slight solvent-induced shifts, the chemical shifts of H-15, H-16, and H-18 are comparable to the corresponding shifts in myricanol (**3**). The difference in the ^{13}C chemical shift of the C-11 oximethine group in myricarborin (δ 77.8) and in myricanol (δ 68.5, recorded in this study) (Joshi et al., 1996, δ 68.7) was indicative of an ether linkage in **1**. Analysis of the HMBC and NOESY spectra enabled us to position the ether function between C-3 and C-11. The proton sequence of the aliphatic ring was made using the ^1H–^1H COSY spectrum and the gradient HMQC spectrum while the HMBC spectrum permitted the construction of the skeleton of myricarborin. In the HMBC spectrum, cross peaks were observed between H-18 (δ 7.04) and C-2 (δ 125.5), C-15 (δ 129.2), C-17 (δ 150.3), and C-19 (δ 125.3). Further HMBC correlations were visible between H-19 (δ 6.70) and C-1 (δ 125.6), C-3 (δ 139.9), and C-5 (δ 110.9). On the basis of all the above data, structure **1** was proposed for myricarborin. This proposed structure was further supported by the NOESY spectrum (see Fig. 2) in which pertinent correlations were observed between H-5 and H-7a, H-7b, and the C-4 methoxy group (δ 3.77); H-15 and H-13a, H-13b, and H-16.

Compound **2** were obtained as colourless needles (CH_2Cl_2), mp 229–231°, $[\alpha]_D^{22} - 46°$, and its molecular formula of $C_{26}H_{34}O_9$ was deduced from the FABMS and 1D NMR spectral data. The IR spectrum indicated absorption bands due to hydroxyl (3400 cm^{-1}) and aromatic ring (1615 and 1500 cm^{-1}) functionalities. The ^1H NMR spectrum was well-resolved and exhibited signals due to two methoxy groups at δ 3.82 and 3.90, and an anomeric proton of a sugar moiety at δ 4.20 (d, $J = 6.5$ Hz). In the ^{13}C-NMR spectrum, the signal of the anomeric carbon of the sugar appeared at δ 100.1 while the other sugar carbons showed up at δ 64.2, 69.4, 72.1, and 74.7 characteristic of D-xylose (Marino-De et al., 1998). The ^1H- and ^{13}C-NMR spectroscopic data of the aglycone part of **2** (Table 1) were almost superimposable on those of myricanol (**3**) (Joshi et al., 1996) also obtained in large quantity in this study. The only significant difference was in the ^{13}C chemical shifts of C-11: δ 75.2 in **2** and δ 68.5 in **3**. The downfield shift observed for this carbon indicated that glycosylation was at C-11. This was confirmed by an HMBC spectral cross peak between the anomeric proton H-1′ (δ 4.20) and C-11 (δ 75.2). The COSY and NOESY spectra also confirm the sugar moiety to be a xylose with a β-linkage. Compound **2** was thus identified as 11-*O*-β-D-xylopyranosylmyricanol. On acid hydrolysis, **2** afforded myricanol (**3**), which was identified by co-TLC with authentic myrica-



1



2 $R_1 = H$; $R_2 = \beta$-D-xylopyranosyl

3 $R_1 = R_2 = H$

4 $R_1 = \beta$-D-glucopyranosyl; $R_2 = H$

Fig. 1. Structures of the isolated compounds.

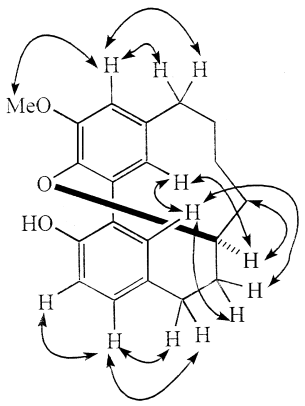


Fig. 2. Pertinent NOESY correlations observed with myricarborin **1**.

nol. Therefore **2** has the same 11R-absolute configuration as **3**.

3. Experimental

3.1. Plant material

The root and stem barks of *M. arborea* Hutch were collected on Mount Bamboutos, West Province, Cameroon, in November 1996. Authentication was done by Mr. Paul Mezili, a retired botanist of the Cameroon National Herbarium, Yaounde. A voucher specimen (PM 1992) has been deposited at the Botany Department, University of Dschang.

3.2. General experimental procedures

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125.8 MHz) were recorded at room temperature in $CDCl_3$ or in $CDCl_3$–CD_3OD using a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in part per million (ppm) with the solvent signals, δ_H 7.26 and δ_C 77.0 as reference, while the coupling constants (J) are given in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The IR spectra were recorded with a Perkin–Elmer 298 spectrophotometer, and the UV spectra recorded with a Varian Cary 2290. EIMS, CIMS, and HREIMS spectra (direct inlet, EI at 70 eV) were recorded with a JEOL SX102 spectrometer, while FABMS were recorded with a JEOL D-300 spectrometer. The melting points (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin–Elmer 141 polarimeter at 22°C. Column chromatography was run on Merck Si gel 60 and gel permeation on Sephadex LH-20, while TLC were carried out on Si gel GF_{254} pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100°C, or by visualizing with an UV lamp at 254 and 366 nm.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (δ;multiplicity; J) for **1**, **2** and **4** in $CDCl_3$–CD_3OD (9:1), with the $CDCl_3$ signal (7.26 and 77.0 ppm) as reference

	^{1}H (δ; multiplicity; J)			^{13}C (δ)		
	1	**2**	**4**	**1**	**2**	**4**
1				125.6	124.6	128.2
2				125.5	123.0	124.1
3				139.9	146.0	146.8
4				147.7	138.9	144.7
5	6.51; *br s*			110.9	148.0	148.2
6				131.2	122.7	130.1
7	2.33/2.77; *m*	2.51/2.74; *m*	2.53/2.73; *m*	25.5	25.5	25.6
8	1.72/1.82; *m*	1.77/1.82; *m*	1.73/1.74; *m*	26.4	25.5	26.1
9	1.39/1.58; *m*	1.49/1.61; *m*	1.40/1.56; *m*	22.6	22.5	22.7
10	1.93/2.07; *m*	1.61/1.82; *m*	1.40/1.73; *m*	36.0	35.1	39.0
11	4.61; *br t*; 9.2	3.98; *br t*; 9.6	3.84; *br t*, 9.8	77.8	75.2	68.1
12	1.78/2.04; *m*	1.82/2.18; *m*	1.56/2.10; *m*	33.0	32.9	34.4
13	2.65/2.85; *m*	2.79/2.85; *m*	2.73/2.74; *m*	30.3	26.7	26.7
14				130.6	131.0	130.9
15	6.87; *dd*; 8.1, 2.1	7.00; *br d*; 8.2	6.94; *dd*; 8.2, 2.0	129.2	129.7	130.2
16	6.65; *d*; 8.1	6.81; *d*; 8.2	6.72; *d*; 8.2	115.9	116.5	116.4
17				150.3	150.9	150.9
18	7.04; *d*; 2.1	7.07; *d*; 2.1	7.02; *d*; 2.0	133.1	132.6	133.0
19	6.70; *br s*	6.79; *s*	6.77; *s*	125.3	129.3	129.0
3-OMe		3.82; *s*	3.84; *s*		61.3	61.4
4-OMe	3.77; *s*	3.90; *s*	3.86; *s*	55.8	61.1	61.2
1′		4.20; *d*; 6.5	4.73; *d*; 7.5		100.1	104.2
2′		3.24; *dd*; 7.7, 6.5	3.42; *t*; 7.6		72.1	74.0
3′		3.37; *t*; 7.7	3.39; *t*; 7.5		74.7	76.2
4′		3.49; *m*	3.40; *t*; 7.3		69.4	69.7
5′		3.67; *dd*; 4.6, 11.8	3.16; *m*		64.2	75.9
6′		2.98; *dd*; 8.5, 11.8	3.16; *m*		64.2	75.9
			3.67; *dd*; 12.2, 3.0			61.4
			3.62; *dd*; 12.2, 7.2			61.4

3.3. Extraction and isolation

The dried powdered plant material (3.5 kg) was extracted successively by percolation with MeOH–CH_2Cl_2 (1:1). The crude organic extract (220 g) was partitioned between hexane and 80% MeOH, and the aqueous MeOH phase diluted with water to 60% MeOH and extracted with CH_2Cl_2. Finally the MeOH layer was further diluted to 50% and exhaustively extracted with EtOAc. Evaporation of the solvent from the hexane, CH_2Cl_2, and EtOAc extracts yielded 52, 70, and 31 g, respectively. The hexane extract was not further examined in this work. A TLC analysis showed that the CH_2Cl_2 and EtOAc extracts were qualitatively the same. They were thus combined, and a portion (50 g) was subjected to CC on Si gel (230–400 mesh) eluting with hexane–EtOAc mixtures of increasing polarity. Fifty-five fractions of 250 ml each were collected and combined on the basis of their TLC profiles to give four major fractions: I (10.5 g, hexane–EtOAc 9:1), II (4.5 g, hexane–EtOAc 4:1), III (3.5 g, hexane–EtOAc 1:1), and IV (18 g, EtOAc). Fractions I and II were shown by TLC (Liebermann–Burchard reagent) to contain mostly triterpenoids. These fractions were therefore not investigated.

Further purification of fractions III and IV was achieved by medium pressure liquid chromatography on a Baeckström AB Separo column (15- mm id) with a continuous gradient of CH_2Cl_2–MeOH. Fraction III yielded myricanol (**3**) (2.5 g) and myricarborin (**1**) (10 mg). Fraction IV afforded 11-*O*-β-D-xylopyranosylmyricanol (**2**) (31 mg), 5-*O*-β-D-glucopyranosylmyricanol (**4**) (800 mg), and a tarry mixture of non-separable products. For each of the compounds, an additional purification by gel permeation CC on Sephadex LH-20 (CH_2Cl_2–MeOH, 1:1) was required to obtain analytically pure samples.

3.4. Myricarborin (*1*)

Colourless plates (CH_2Cl_2), m.p. 141–143°. $[\alpha]_D^{22}$ + 9.3° (c 0.41, MeOH); Analysis: found: C, 77.39; H, 7.14. $C_{20}H_{22}O_3$ requires: C, 77.40; H, 7.15%; EIMS (probe) 70 eV (rel. int.) *m/z*: 310 $[M]^+$ (100), 264 (8), 242 (15), 277 (12), 185 (5), 165 (5), 121 (8). CIMS (CH_4) 325 $[M + CH_3]^+$ (2), 311 $[M + H]^+$ (100), 297 (3), 279 (3), 269 (6). UV (MeOH) λ_{max} (log ε) nm: 214 (4.32), 256 (3.82), 328 (3.71). IR (KBr) ν_{max} cm^{-1}: 3420 (OH), 2930, 1620, 1610 (C=C), 1500, 1410, 1385, 1235, 1065, 1045, 925, 800; ^{1}H-NMR ($CDCl_3$–CD_3OD, 500 MHz) see Table 1; ^{13}C-NMR ($CDCl_3$–CD_3OD, 125 MHz) see Table 1.

3.5. 11-O-β-D-Xylopyranosylmyricanol (*2*)

Colourless needles (CH_2Cl_2), m.p. 229–231°, $[\alpha]_D^{22}$ – 46° (c 0.55, MeOH); Analysis: found: C, 63.66; H, 6.99. $C_{26}H_{34}O_9$ requires: C, 63.65; H, 7.00%; FABMS *m/z*: 513 $[M + Na]^+$, 491 $[M + H]^+$ UV (MeOH) λ_{max} (log ε) nm: 214 (4.46), 260 (3.97), 298 (3.79); IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2930, 1615 (C=C), 1500, 1450, 1230, 1065, 1040; ^{1}H-NMR ($CDCl_3$–CD_3OD, 500 MHz) see Table 1. ^{13}C-NMR ($CDCl_3$–CD_3OD, 125 MHz) see Table 1.

3.6. Acid hydrolysis of *2*

Compound **2** (2 mg) was heated with 10% H_2SO_4 (4 ml) at 100° for 30 min. The reaction mixture was extracted with CH_2Cl_2 and the extract concentrated to give myricanol (**3**) (1 mg), which was identified by mixed melting point and co-TLC.

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