Two New Macrocyclic Diaryl Ether Heptanoids from *Boswellia ovalifoliolata*[1)]

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The stems of *Boswellia ovalifoliolata* Bal. & Henry (Burseraceae) afforded two new macrocyclic diaryl ether heptanoids, ovalifoliolatin A (1) and B (2) together with three known compounds; acerogenin C (3), 3α-hydroxyurs-12-ene (4), and sitost-4-en-3-one (5). The structures were established by means of spectroscopic analysis and compounds 1, 3—5 were evaluated for their antibacterial activity.

Key words *Boswellia ovalifoliolata*; Burseraceae; diaryl ether heptanoid; ovalifoliolatin A; ovalifoliolatin B; antibacterial activity

The diarylheptanoids belong to a distinct class of naturally occurring compounds, featured by the characteristic presence of two aromatic rings tethered by a linear seven carbon chain. These are further divided into three subgroups, namely linear diarylheptanoids, macrocyclic biaryl heptanoids, and macrocyclic diaryl ether heptanoids.[2—4)] This class of diaryl heptanoids exhibits a broad range of potent biological activities that include anti-inflammatory, antihepatotoxic, antifungal, antibacterial, and related effects.[5)] Recently, several of these compounds were found to inhibit nitric oxide (NO) production in LPS-activated mouse peritoneal macrophages.[6,7)]

During the course of our chemo-taxonomic studies on medicinal plants of the Indian System of Medicine (ISM),[8,9)] a detailed chemical investigation of the stem of *Boswellia ovalifoliolata* Bal. & Henry (Burseraceae) afforded two new macrocyclic diaryl ether heptanoids named ovalifoliolatin A (**1**) and B (**2**) together with the known macrocyclic diaryl ether heptanoid acerogenin C (**3**),[10,11)] a known triterpenoid, 3α-hydroxyurs-12-ene (**4**),[12,13)] and a known steroid, sitost-4-en-3-one (**5**).[14)] These structures were established by means of spectroscopic analysis and an evaluation of the antibacterial activity of the isolated compounds was conducted.

Results and Discussion

Ovalifoliolatin A (**1**) was obtained as a semisolid, $[\alpha]_D^{25}$ −8.88° (c=0.65, MeOH) and its molecular formula $C_{20}H_{20}O_4$ was established by HR-FAB-MS at m/z 324.3767 (calcd. 324.3774). Its IR spectrum showed bands at 3479 and 1715 cm^{-1} for the presence of hydroxyl and keto carbonyl groups. Its ^{1}H-NMR spectrum displayed seven aromatic proton signals ascribable to two benzene rings *viz*, a 1,2,5-trisubstituted benzene at δ 6.72 (1H, d, J=8.2 Hz, H-3), 6.60 (1H, dd, J=2.0, 8.2 Hz, H-4) and 4.93 (1H, d, J=2.0 Hz, H-6) and a 1,4-disubstituted benzene at δ 7.48 (1H, dd J=2.1, 8.2 Hz, H-15); 7.08 (1H, dd, J=2.2, 8.2 Hz, H-16); 7.03 (1H, dd, J=2.2, 8.2 Hz, H-18) and 7.32 (1H, dd, J=2.1, 8.2 Hz, H-19). Its ^{1}H-NMR spectrum further displayed signals due to a disubstituted trans olefine at δ 6.28 (1H, d, J=15.5 Hz, H-7) and 5.02 (1H, m, H-8), a methine proton bearing a hydroxyl group at δ 4.42 (1H, br s, H-12) and three methylene groups at δ 2.44 (1H, m, H_a-9) and 2.27 (1H, m, H_b-9); 2.85 (1H, m, H_a-10) and 2.36 (1H, m, H_b-10) and 3.20 (1H, dd, J=6.2, 13.9 Hz, H_a-13) and 3.39 (1H, dd, J=3.5, 13.9 Hz, H_b-13) respectively. Further, its ^{13}C-NMR spectrum showed twenty carbon signals (Table 1) and characteristic signals of diaryl ether heptanoid.[5,15)] The signal at δ 4.93 was attributed to the H-6 aromatic proton, which generally resonates abnormally at high field between δ 4.9—6.1 (d, J=2 Hz), owing to the anisotropic effect of the B-ring in diaryl ether heptanoids,[16)] which was further confirmed from its dreiding models and using a semiempirical AM1 method[17)] (Fig. 2). The dispositions of the hydroxyl, the disubstituted double bond and the keto group were determined by its ^{1}H–^{1}H- correlation spectroscopy (COSY) (Fig. 1), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) (Table 1) experiments. In the HMBC spectrum of **1**, the C-13 methylene protons at δ 3.20 and 3.39 showed correlations with C-11 (δ 210.76, s), C-12 (δ 77.26, d), C-14 (δ 132.59, s), C-15 (δ 131.66, d), and C-19 (δ 132.32, d) carbons and the C-12 methine proton bearing a hydroxy group at δ 4.42 showed correlations with carbons at C-11 (δ 210.76, s), C-13 (δ 38.50, t), and C-14 (δ 132.59, s), respectively. Similarly, the C-7 olefinic proton at δ 6.28 showed correlations with carbons at C-4 (δ 118.81, d), C-6 (δ 118.0, d), C-8 (δ 134.43, d), and C-9 (δ 24.97, t). The C-8 olefinic proton at δ 5.02 showed correlations with carbons at C-5 (δ 133.71, s), C-7 (δ 128.8, d), C-9 (δ 24.97, t), and C-10 (δ 39.88, t), respectively. The relative stereochemistry of the C-12 hydroxyl group was established by its nuclear Overhauser enhancement spectroscopy (NOESY) (Fig. 1) spectrum. A careful study of its NOESY spectrum revealed that the H-6 aromatic proton δ 4.93 (1H, d, J=2 Hz) showed correlations with the H-8, H-16, and H-18 protons. Further, the C-13 methylene protons at δ 3.39 (1H, dd, J=3.5, 13.9 Hz, H_a-13) and 3.20 (1H, dd, J=6.2, 13.9 Hz, H_b-13) showed correlations with H-19 and H-15, respectively. The H-12 proton at δ 4.42 (1H, br s) showed strong correlations with H-19 and H_a-13; and weak correlation with H_b-13. The foregoing spectral data envisages that an –OH group at C-12, a bulkier radical than a hydrogen atom,

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Table 1. Spectral Data of Ovalifoliolatin A (**1**) and B (**2**)

Position	Ovalifoliolatin A (**1**)			Ovalifoliolatin B (**2**)
	^{1}H-NMR[a] Multiplicity (J in Hz)	^{13}C-NMR & DEPT[b]	HMBC[c] (J=7 Hz)	^{1}H-NMR[a] Multiplicity (J in Hz)
C-1		146.96		
C-2		151.54		
C-3	6.72, 1H, d (8.2, H-3)	111.37	C-1, C-2, C-4, C-5, C-6	6.73, 1H, d (8.2)
C-4	6.60, 1H, dd (2.0, 8.2, H-4)	118.81	C-1, C-2, C-6, C-7	6.60, 1H, dd (2.1, 8.2)
C-5		133.71		
C-6	4.93, 1H, d (2.0, H-6)	118.0	C-1, C-2, C-4, C-5, C-7	4.96, 1H, d (2.1)
C-7	6.28, 1H, d (15.5, H-7)	128.8	C-4, C-6, C-8, C-9	6.23, 1H, d (15.2)
C-8	5.02 (1H, m, H-8)	134.43	C-5, C-7, C-9, C-10	5.16, 1H, m
C-9	2.44 (1H, m, H_a-9)	24.97	C-7, C-8, C-10, C-11	2.33, 2H, m
	2.27 (1H, m, H_b-9)			
C-10	2.85 (1H, m, H_a-10)	39.88	C-8, C-9, C-11	2.38, 2H, m
	2.36 (1H, m, H_b-10)			
C-11		210.76		
C-12	4.42 (1H, br s, H-12)	77.26	C-11, C-13, C-14	2.77, 2H, m
C-13	3.39, 1H, dd (3.5, 13.9, H_a-13)	38.50	C-11, C-12, C-14, C-15, C-19	3.03, 2H, m
	3.20, 1H, dd (6.2, 13.9, H_b-13)			
C-14		132.59		
C-15	7.48, 1H, dd (2.1, 8.2, H-15)	131.66	C-13, C-14, C-17	7.37, 1H, d (8.4)
C-16	7.08, 1H, dd (2.2, 8.2, H-16)	124.15	C-15, C-17, C-18	7.05, 1H, d (8.4)
C-17		157.0		
C-18	7.03, 1H, dd (2.2, 8.2, H-18)	124.64	C-16, C-17, C-19	7.05, 1H, d (8.4)
C-19	7.32, 1H, dd (2.1, 8.2, H-19)	132.32	C-13, C-14, C-17	7.37, 1H, d, (8.4)
C-20	3.94 (3H, s, OMe)	56.13		3.94 (3H, s, OMe)

a) Measured in $CDCl_3$, 400 MHz; *b*) Measured in $CDCl_3$, 75 MHz; *c*) Measured in $CDCl_3$, 500 MHz.

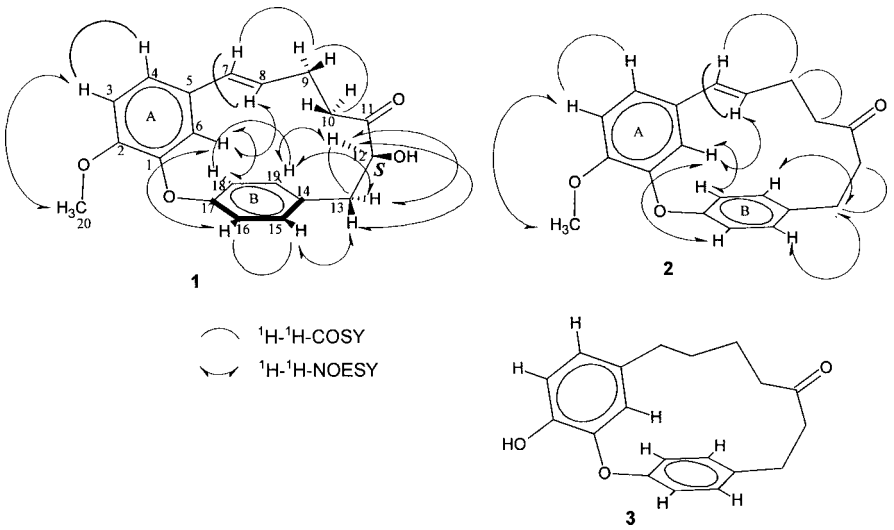
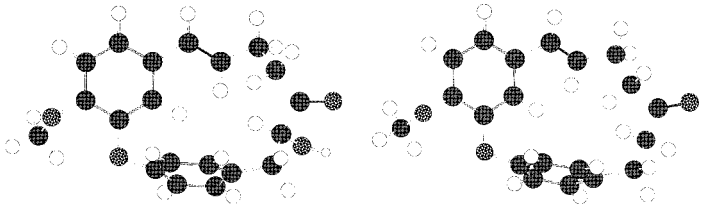


Fig. 1

should be directed away from the inside of the macrocyclic ring and the configuration of 12-OH causes the different chemical shifts between H_a-13 & H_b-13; H-15 & H-19; and H-16 & H-18. Therefore, the relative stereochemistry at C-12 was established as a β configuration, which was further confirmed by a semiempirical AM1 method, and the relative energy ordering is not correctly reproduced at the MM2 level of theory. The AM1 optimized geometries (Fig. 2) were employed in PCMODEL package[18] to obtain coupling constants, which are in good agreement with the experimental values ($J_{13,12}$). Thus, the forgoing spectral data confirmed the structure of ovalifoliolatin A as **1**.

Ovalifoliolatin B (**2**) was obtained as an optically inactive semisolid in a small quantity (3 mg). Its molecular formula, $C_{20}H_{20}O_3$, was established by HR-FAB-MS at m/z 308.3772 (calcd. 308.3781). The IR spectrum showed a band at 1711 cm^{-1} for the presence of a keto carbonyl group. The ^{1}H-NMR spectrum of ovalifoliolatin B is similar to that of ovalifoliolatin A except for the absence of a signal due to a hydroxy bearing methine proton at δ 4.42 (H-12).

Its ^{1}H-NMR spectrum displayed seven aromatic proton signals for the presence of two benzene rings *viz*, a 1,2,5-trisubstituted benzene at δ 6.73 (1H, d, J=8.2 Hz, H-3), 6.60 (1H, dd, J=2.1, 8.2 Hz, H-4) and 4.96 (1H, d, J=2.1 Hz, H-6), and a 1,4-disubstituted benzene at δ 7.37 (2H, d, J=8.4 Hz, H-15, H-19) and 7.05 (2H, d, J=8.4 Hz, H-16, H-



1 2

Fig. 2

Table 2. Antibacterial Activity of Compounds **1**, **3**—**5**

	Name of the organism	Concentration of compound (μg/disk)[a]												Kanamycin[b] (30 μg/disk)
		1			**3**			**4**			**5**			
		25	50	100	25	50	100	25	50	100	25	50	100	
Gram positive	*S. aureus*	—	9	16	8	9	10	7	8	11	7	8	8	10
	B. subtilis	10	11	13	9	9	12	8	9	11	11	12	13	18
	B. sphaericus	7	11	14	7	8	10	—	7	10	12	12	13	20
Gram negative	*C. violaceum*	—	7	17	8	9	11	9	10	11	—	—	—	17
	K. aerogenes	7	8	12	9	10	11	9	10	12	10	11	12	15
	P. aeruginosa	8	9	13	8	9	11	9	10	12	10	11	12	27
	P. flourescens	—	—	—	7	7	9	8	9	12	—	—	—	15

a) Values are shown in zone of inhibition in mm/dia. *b*) Positive control.

18). Further, its ^{1}H-NMR spectrum displayed signals due to a disubstituted *trans*-olefine at δ 6.23 (1H, d, J=15.2 Hz, H-7) and 5.16 (1H, m, H-8), and four methylene groups at δ 2.33 (2H, m, H-9), 2.38 (2H, m, H-10), 2.77 (2H, m, H-12), and 3.03 (2H, m, H-13). The upfield chemical shift for H-6 is due to an anisotropic effect of the B-ring in diaryl ether heptanoids[16] as noticed in compound **1**, which was further confirmed from its dreiding models and using a semiempirical AM1 method[16] (Fig. 2). These characteristics indicated that compound **2** is a diaryl ether heptanoid.[5,16] The disposition of the keto group and the disubstituted double bond was confirmed by its ^{1}H–^{1}H-COSY and ^{1}H–^{1}H-NOESY (Fig. 1) spectral data. In the ^{1}H–^{1}H-COSY spectrum of **2**, a linear connectivity has been observed from the H-7 to H-10 protons. Further, H-13 methylene protons at δ 3.03 showed correlation with H-12 methylene protons at δ 2.77. Thus, the foregoing spectral data confirmed the structure of ovalifoliolatin B (**2**) as a 12-dehydroxy derivative of **1**. This is the first report of the occurrence of diaryl ether heptanoids from *Boswellia* sp. and hitherto no chemical examination has been reported on this plant.

Antibacterial Activity The antibacterial activity was assayed by disk susceptibility tests according to the NCCLS (Wayne, 1997)[19] and as described in our previous paper.[3] Inocula were adjusted to 0.5 McFarland turbidity. Excess moisture was allowed to absorb for 10 min before applying dried disks containing the compound. Compounds were dissolved in sterile DMSO, which did not influence the growth of bacteria. Kanamycin was used as a positive control according to the standard method. The test plates were incubated at 37 °C and zones of inhibition were recorded after 24 h. All the compounds were tested, except compound **2**, against gram-negative bacteria *Chromobacterium violaceum* (ATCC # 12472), *Klebsiella aerogenes* (ATCC # 15380), *Pseudomonas aeruginosa* (ATCC # 25619) and *Pseudomonas flourescens* (ATCC # 13525) and gram-positive bacteria *Staphylococcus aureus* (ATCC # 9144), *Bacillus subtilis* (ATCC # 6051), and *Bacillus sphaericus* (ATCC #14577).

Compound **1** is highly active (Table 2) against *S. aureus* and *C. violaceum* and moderately active against *K. aerogenes*, *B. subtilis*, *P. aeruginosa* and *B. sphaericus*. The remaining compounds are moderately active against all the organisms. Interestingly, gram-positive bacteria are highly susceptible to compound **1** when compared with the positive control kanamycin.

Experimental

General The ^{1}H-NMR and 2D spectra (^{1}H–^{1}H-COSY, ^{1}H–^{1}H-NOESY) were recorded on a Varian Unity INOVA 400 MHz spectrometer with standard pulse sequences, and ^{13}C-NMR spectra were recorded on an Avance Brucker 300 MHz. Silica gel (100—200 mesh, ACME) was used for open column chromatography. UV and IR spectra were recorded on Shimadzu 240 and Perkin-Elmer RXI FT-IR spectrophotometers, respectively. Mass spectra were recorded on a Finnigan-MAT 1020 instrument. Optical rotations were measured on a JASCO DIP-370 polarimeter.

Plant Material The plant material of *Boswellia ovalifoliolata* (Burseraceae) was collected from Seshachalam Hill ranges (Tirumula forest) of the Eastern Ghats in Andhra Pradesh, India during October 2001 and was identified by Prof. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India.[20] The voucher specimen (No. 2076) is kept in the herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, India.

Extraction and Isolation The shade dried and powdered (1.8 kg) stem part of the plant *Boswellia ovalifoliolata* was extracted with *n*-hexane (3×4.5 l) followed by CH_2Cl_2 : MeOH (1 : 1, 3×4.5 l) at room temperature. The combined hexane extract was filtered and concentrated under reduced pressure to obtain a greenish gummy residue (10.2 g). The concentrated *n*-hexane extract was subjected to silica gel column chromatography by gradient elution using a CCl_4–EtOAc gradient to afford 3α-hydroxyurs-12-ene (**4**, 30 mg) and sitost-4-en-3-one (**5**, 18 mg). The combined CH_2Cl_2 : MeOH extract was filtered and concentrated under reduced pressure to obtain a greenish gummy residue (47.5 g) which was subjected to silica gel column chromatography eluted with hexane–EtOAc gradient to afford two fractions A and B, which were further subjected to silica gel column chromatography eluted with CCl_4/EtOAc (9.8 : 0.2) and CCl_4/EtOAc (8 : 2) respectively, to af-

ford ovalifoliolatin B (**2**, 3 mg) and acerogenin C (**3**, 25 mg) from fraction A and ovalifoliolatin A (**1**, 80 mg) from fraction B.

Ovalifoliolatin A (**1**): Obtained as a semisolid; $[\alpha]_D^{25}$ −8.88° (c=0.65, MeOH); IR (KBr) ν_{max}: 3479 (OH), 3019, 2932, 2847, 1715 (C=O), 1682, 1651, 1514, 1505, 1442, 1414, 1269, 1223, 1124, 1025, 965, 837, 754, 666, 619. UV λ_{max} (MeOH) nm (log ε): 207 (4.09), 261 (3.73). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): (see Table 1). ^{13}C-NMR ($CDCl_3$, 75 MHz, δ): (see Table 1) HR-FAB-MS, obsd. m/z 324.3767 [M^+, $C_{20}H_{20}O_4$] requires m/z 324.3774. FAB-MS obsd. m/z (%): 324 (M^+, 47), 295 (10), 253 (11), 221 (15), 189 (16), 159 (35), 146 (57).

Ovalifoliolatin B (**2**): Obtained as a semisolid; $[\alpha]_D^{25}$ 0.0° (c=0.15, $CHCl_3$); IR (KBr) ν_{max}: 2931, 2831, 2857, 1711 (C=O), 1592, 1502, 1429, 1404, 1365, 1259, 1190, 1109, 1079. UV λ_{max} (MeOH) nm (log ε): 208 (3.83), 260 (3.30). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): (see Table 1). HR-FAB-MS, obsd. m/z 308.3772 [M^+, $C_{20}H_{20}O_3$] requires m/z 308.3781. FAB-MS obsd. m/z (%): 308 (M^+, 7), 283 (7), 257 (11), 136 (22), 123 (27), 109 (41), 95 (61), 81 (64), 69 (71), 57 (100).

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