

ADDITIONAL BIOACTIVE NEOLIGNANS FROM THE ROOTS OF *ENDLICHERIA DYSODANTHA*

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**Abstract**— Three additional new neolignans, dysodanthins D, E, and F, and one known neolignan, megaphyllone acetate, have been isolated from the ethanol extract of the roots of *Endlicheria dysodantha* (Lauraceae). Their structures were solved using spectral data. Results of biological testing suggest further evaluation of megaphyllone acetate as an antitumor agent.

Bioactivity directed fractionation, using the brine shrimp lethality assay,<sup>1,2</sup> the inhibition of crown gall tumors on potato discs,<sup>2,3</sup> and human tumor cell cytotoxicities, previously led to the isolation of four benzylbenzoates<sup>4</sup> and four neolignans (megaphone acetate, dysodanthins A-C)<sup>5</sup> from the roots of *Endlicheria dysodantha* Mez. (Lauraceae). As part of our continuing investigation of the bioactive constituents of this plant, four additional neolignans (1-4) have been isolated from the EtOH extract. All of these neolignans showed cytotoxic effects to human solid tumor cells. One of these, megaphyllone acetate (1), showed significant activities in the simple bioassays,<sup>1-3</sup> and cytotoxicities comparable to adriamycin against human colon tumor cells, suggesting a good potential for animal *in vivo* antitumor effects. Another compound, dysodanthin E(3), showed borderline, but yet significant, cytotoxicity against human breast tumor cells.

Compound (1) was isolated as a yellow oil (52 mg; 0.0026%) with  $[\alpha]_D = 0^\circ$  (c=0.1, MeOH). High resolution eims showed the molecular formula  $C_{23}H_{28}O_7$  (found 416.4751, calcd 416.4754). The ir spectra of 1 showed an ester carbonyl peak at  $1740\text{ cm}^{-1}$  and an  $\alpha, \beta$ -unsaturated carbonyl peak at  $1673\text{ cm}^{-1}$ , and this was supported by the peaks at 169.64 and 201.19 ppm in the  $^{13}\text{C}$  nmr spectra (Table 1.). The singlet at 5.94 ppm (2H) and two doublets at 6.50 ppm ( $J = 1.5$ ) and 6.57 ppm ( $J = 1.5$ ) in the  $^1\text{H}$  nmr spectra (Table 1.) indicated the presence of a methylenedioxyphenyl group. An allyl group was indicated in the  $^1\text{H}$  nmr with peaks at 2.31, 5.57, 5.01, and 5.02 ppm. Compound (1), thus, was considered as another neolignan. By spectral comparison with the known neolignan, megaphone acetate,<sup>3</sup> this compound was identified as megaphyllone acetate which was previously isolated from *Aniba megaphylla*.<sup>6</sup> Brine shrimp lethality:  $\text{LC}_{50}$  424 ppm; potato disc assay: T/C 42% tumor inhibition; human tumor cell cytotoxicities  $\text{ED}_{50}$  values: A-549 (lung)<sup>7</sup>  $2.26 \times 10^{-1}$   $\mu\text{g/ml}$ , MCF-7 (breast)<sup>8</sup>  $4.02 \times 10^{-1}$   $\mu\text{g/ml}$ , and HT-29 (colon)<sup>9</sup>  $4.10 \times 10^{-2}$   $\mu\text{g/ml}$ .  $\text{ED}_{50}$  values of adriamycin (as a positive control):  $2.92 \times 10^{-2}$   $\mu\text{g/ml}$  for A-549;  $1.15 \times 10^{-1}$   $\mu\text{g/ml}$  for MCF-7; and  $3.44 \times 10^{-2}$   $\mu\text{g/ml}$  for HT-29.

Dysodanthin D (2) was isolated as a colorless oil (30 mg; 0.0015%) with  $[\alpha]_D = -29^\circ$  (c=0.1, MeOH). High resolution eims suggested the molecular formula  $C_{22}H_{32}O_7$  (found 408.2144, calcd 408.2148). The ir spectra indicated the presence of an OH group at  $3414\text{ cm}^{-1}$  but no carbonyl group. The  $^1\text{H}$  nmr spectra (Table 1) showed the familiar allyl peaks at 2.34 ppm (1H, dd,  $J = 14.1, 8.1$ ), 2.53 ppm (1H, dd,  $J = 7.1, 14.1$ ), 5.93 (1H, m), 5.03 ppm (1H, dd,  $J = 16.9, 1.7$ ), and 5.01 ppm (1H, dd,  $J = 10.8, 1.7$ ). Three OMe peaks at 3.85 ppm (3H, s) and 3.48 ppm (6H, s) in the  $^1\text{H}$  nmr spectra suggested they were attached on the aromatic ring; one OMe peak at 3.05 ppm (3H, s) indicated that it was linked to an aliphatic carbon. The singlet peak at 6.51 ppm (2H, s) indicated that the substitution of the aromatic ring was symmetrical. Ir and  $^{13}\text{C}$  nmr spectra (Table 2) showed no carbonyl group in this molecule, but an unusual downfield quaternary carbon at 105.57 ppm which, when considered with the presence of two OH groups as indicated by eims, suggested the presence of a furan ring. Therefore, compound (2) was proposed to be a hydrobenzofuran neolignan. The  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra indicated a *cis*-relationship of the vicinal C-methyl group (0.57 ppm) with the aryl group and a *trans*-relationship of the C-allyl group (40.34 ppm) with the methyl group. Acetylation of 2 gave a monoacetate with a molecular ion at  $m/z$  450 in the cims. The H6 of the monoacetate of 2 was shifted downfield by 1.5 ppm, and a new methyl peak was evident at 1.82 ppm in the  $^1\text{H}$  nmr spectra which indicated the OH group at C6 was acetylated in the monoacetate. In order to eliminate the possibility of a formula isomer, i.e., 5-OH, 6-OMe vs. 6-OH, 5-OMe, long-range HETCOR was performed. Two  $^3J$  couplings were observed in the L-HETCOR spectra: H4 with C8

and H4 with C6, and, combining this as a part of the structure of **2** with the information from the COSY spectra, the moiety of  $\text{CH}_2\text{CHOMeCHOHCH}_2$  was confirmed. The relative stereochemistry of **2** was determined by comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  nmr data with those of porosa **5a**<sup>10</sup> which has a similar structure to **2**. Thus, dysodanthin D (**2**) was identified as rel-(2R, 3S, 3aR, 5R, 6R, 8S)-3a-allyl-5-methoxy-2-(3', 4', 5'-trimethoxy)-3-methyl-2, 3, 3a, 4, 5, 6, 7, 8-octahydrobenzofuran. This is a new natural compound. The bioactivities of this compound are not significant:  $\text{LC}_{50}$  for brine shrimp lethality >1000 ppm; potato disc assay: T/C 11% tumor inhibition;  $\text{ED}_{50}$  values of human tumor cell cytotoxicities: 41.77  $\mu\text{g/ml}$  for A-549; 32.60  $\mu\text{g/ml}$  for MCF-7, and 32.10  $\mu\text{g/ml}$  for HT-29.

Dysodanthin E (**3**) was isolated as a colorless oil (9 mg; 0.0005%) with  $[\alpha]_{\text{D}} = +11^\circ$  ( $c=0.1$ , MeOH). The high resolution eims spectra showed the molecular formula  $\text{C}_{22}\text{H}_{32}\text{O}_7$  (found 408.2148, calcd 408.2148). The eims fragmentation of **3** was identical to that of **2**, and ir and  $^1\text{H}$  nmr spectra (Table 1.) of **3** were very similar to those of **2**. By comparison of the  $^{13}\text{C}$  nmr spectra of **3** (Table 2.) with those of **2**, **3** was considered as an isomer of **2**, with the OH at C6 being axial instead of equatorial. Indeed, acetylation with acetic anhydride-pyridine at room temperature led to the mixture of an  $\alpha$ ,  $\beta$ -unsaturated ketone (megaphone acetate) and the expected monoacetate which were indicated by the molecular ions at  $m/z$  390 and 450. When the reaction was carried out at reflux temperature, the major product was megaphone acetate which was indicated by molecular ion at  $m/z$  432, and with the peaks at  $m/z$  389, 266, 224, and 192 in the eims spectra. Therefore, the OH of **3** must be axial, and, hence, the hemiketal of **3** had been opened and lost a molecule of  $\text{H}_2\text{O}$  through a *trans*-elimination to form megaphone acetate. By comparison of the spectral data with those of porosa **5b**,<sup>11</sup> which has a similar structure, the structure of compound **3**, thus, was identified as rel-(2R, 3S, 3aR, 5R, 6S, 8S)-3a-allyl-5-methoxy-6, 8-dihydroxy-2-(3', 4', 5'-trimethoxy)-3-methyl-2, 3, 3a, 4, 5, 6, 7, 8-octahydrobenzofuran. **3** is also new to the literature. It showed, marginally significant  $\text{ED}_{50}$  values to human tumor cells: 9.86  $\mu\text{g/ml}$  for A-549, 3.32  $\mu\text{g/ml}$  for MCF-7, and 4.14  $\mu\text{g/ml}$  for HT-29;  $\text{LC}_{50}$  of brine shrimp lethality: >1000 ppm; potato discs assay: T/C 8% tumor inhibition.

Dysodanthin F (**4**) was isolated as a colorless oil (125 mg; 0.0063%) with  $[\alpha]_{\text{D}} = -21^\circ$ , ( $c=0.15$ , MeOH). Its high resolution eims indicated the molecular formula  $\text{C}_{23}\text{H}_{34}\text{O}_7$  (found 422.2297, calcd 422.2305). The ir spectrum of **4** was very similar to those of **2** and **3**. The  $^1\text{H}$  nmr spectra showed two aliphatic methoxy peaks at 3.10 ppm and 3.19 ppm instead of one as in **2** and **3**. Since the  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectral data indicated that the

aromatic portion, the furan ring, and the allyl group were the same as in **2** and **3**, the remaining question was the position of the second methoxy group. Further investigation of the  $^{13}\text{C}$  nmr spectrum, which was assigned with the help of HETCOR, found that the chemical shifts of C8 (106.94 ppm) and C7 (32.43 ppm) in **3** were moved to 104.87 ppm and 37.77 ppm in **4**, and the chemical shift of C6 was not changed. Therefore, the second methoxy group of **4** was substituted at C8; this conclusion also was supported by the unusually upfield chemical shift (47.93 ppm) of the methoxy peak due to the stereo restriction. By comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra with those of **2** and **3**, dysodanthin F (**4**) was identified as rel-(2R, 3S, 3aR, 5R, 6S, 8S)-3a-allyl-5, 8-dimethoxy-6-hydroxy-2-(3', 4', 5'-trimethoxy)-3-methyl-2, 3, 3a, 4, 5, 6, 7, 8-octahydrobenzofuran. This neolignan is also new to the literature. It was not significantly bioactive:  $\text{LC}_{50}$  for brine shrimp lethality: >1000 ppm; potato disc assay: T/C 13% tumor inhibition;  $\text{ED}_{50}$  values of human tumor cell cytotoxicities: 54.57  $\mu\text{g}/\text{ml}$  for A-549, 53.31  $\mu\text{g}/\text{ml}$  for MCF-7, and 38.12  $\mu\text{g}/\text{ml}$  for HT-29.

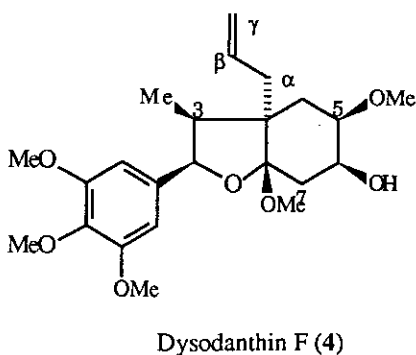
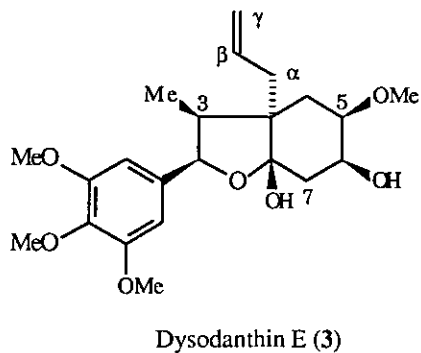
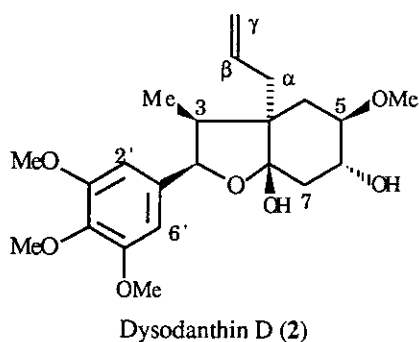
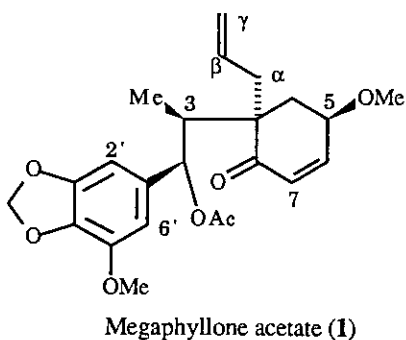


TABLE 1 <sup>1</sup>H Nmr Data of Compounds 1-4 (500 MHz).

Proton	Compound δ H (J, Hz)			
	1 (CDCl <sub>3</sub> )	2 (C <sub>6</sub> D <sub>6</sub> )	3 (C <sub>6</sub> D <sub>6</sub> )	4 (C <sub>6</sub> D <sub>6</sub> )
2	5.65 s	5.27 d (9.8)	5.28 d (9.9)	5.04 d (9.8)
3	2.52 q (7.2)	2.78 dq (9.8, 7.4)	2.77 dq (9.9, 7.3)	2.68 dq (9.8, 7.4)
4a	1.86 dd (10.0, 13.2)	1.77 dd (4.1, 13.4)	1.56 dd (13.3, 3.5)	1.56 dd (13.2, 3.8)
4b	2.30 ddd (13.2, 5.2, 1.8)	1.44 dd (12.5, 13.4)	1.93 dd (13.3, 13.0)	1.96 dd (12.7, 13.2)
5	4.21 dddd (10.0, 5.2, 2.1, 1.9)	3.25 m	3.19 m	3.17 m
6	6.91 dt (10.4, 1.9)	4.16 m	3.97 m	4.00 m
7a	6.02 dd (10.4, 2.1)	2.06 dd (13.9, 12.8)	1.75 dd (15.1, 3.4)	1.37 m
7b		2.72 dd (5.2, 13.9)	2.47 dd (15.1, 2.5)	1.37 m
α	2.36 d (7.1)	2.34 dd (14.1, 8.1)	2.53 dd (14.4, 7.1)	2.54 dd (14.1, 7.1)
α'	2.36 d (7.1)	2.53 dd (7.1, 14.1)	2.23 dd (14.4, 8.4)	2.59 dd (14.1, 3.0)
β	5.57 ddt (16.8, 10.4, 7.1)	5.93 m	5.92 m	5.91 m
γ	5.01 dd (16.8, 1.7)	5.03 dd (16.9, 1.7)	5.06 dd (16.8, 1.1)	5.06 dd (16.9, 1.1)
γ'	5.02 dd (10.4, 1.7)	5.01 dd (10.8, 1.7)	5.00 dd (10.1, 1.1)	5.00 dd (10.6, 1.1)
2'	6.50 d (1.5)	6.51 s	6.80 s	6.83 s
6'	6.57 d (1.5)	6.51 s	6.80 s	6.83 s
5-OMe	3.45 s	3.05 s	3.17 s	3.10 s
8-OMe				3.19 s
3',5'-OMe	3.93 s	3.48 s	3.55 s	3.54 s
4'-OMe		3.85 s	3.84 s	3.85 s
OCH <sub>2</sub> O	5.94 s			
OCOMe	2.11 s			
3-Me	0.91 d (7.2)	0.57 d (7.4)	0.61 d (7.3)	0.63 d (7.4)

TABLE 2.  $^{13}\text{C}$  Nmr Data of Compounds 1-4 (125 MHz).

Carbon	Compound $\delta$ C			
	1 ( $\text{CDCl}_3$ )	2 ( $\text{C}_6\text{D}_6$ )	3 ( $\text{C}_6\text{D}_6$ )	4 ( $\text{C}_6\text{D}_6$ )
2	75.53	80.98	81.89	81.93
3	41.16	44.53	44.37	44.52
3a	52.17	49.41	49.45	50.28
4	34.52	30.49	27.55	27.60
5	73.19	82.14	77.52	77.44
6	147.74	71.23	66.39	65.96
7	128.97	39.65	37.77	32.43
8	201.19	105.57	104.87	106.94
$\alpha$	38.70	40.34	39.32	39.06
$\beta$	132.33	136.01	136.09	136.22
$\gamma$	118.31	117.38	117.29	117.15
1'	134.01	136.01	136.09	138.17
2'	99.52	104.56	104.85	104.89
3'	148.48	153.82	153.83	153.89
4'	136.37	136.11	137.93	135.84
5'	143.30	153.82	153.83	153.89
6'	104.99	104.56	104.85	104.89
5-OMe	56.57	56.62	56.03	55.86
8-OMe				47.93
3',5'-OMe	56.37	55.82	55.91	55.95
4'-OMe		60.56	60.57	60.56
$\text{OCH}_2\text{O}$	101.30			
$\text{OCOMe}$	169.64			
$\text{OCOMe}$	21.33			
3-Me	5.78	12.17	12.17	12.29

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