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BIOACTIVE NEOLIGNANS FROM *ENDLICHERIA DYSODANTHA*

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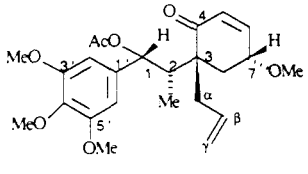
ABSTRACT.—From bioactivity-directed fractionation of the EtOH extract of *Endlicheria dysodantha*, dysodanthin A [**2**] and dysodanthin B [**3**], which are new hexahydrobenzofuranoid neolignans, have been isolated. In addition, the known neolignans, compound **4**, which is a burchellin analogue, and megaphone acetate [**1**] were isolated. All four neolignans showed activities in the brine shrimp lethality test; compounds **1–3** also inhibited the growth of crown gall tumors on potato discs and were cytotoxic to human tumor cells in culture. This is the first report of these neolignans isolated from the genus *Endlicheria* and of their completely assigned ^{1}H- and ^{13}C-nmr data.

In a previous paper on *Endlicheria dysodantha* Mez. (Lauraceae), the occurrence of four bioactive benzyl benzoates was described (1). We have now used bioactivity-directed fractionation, with the brine shrimp lethality test (2), to isolate four bioactive neolignans from the EtOH extract of the roots of this plant. All of these neolignans were active in the brine shrimp lethality test (1), and compounds **1–3** also inhibited crown gall tumors on potato discs (3) and were cytotoxic to human tumor cells (Table 1). Neolignans are widespread constituents among the Lauraceae family (4,5). However, this is the first report of bioactive neolignans isolated from the genus *Endlicheria*. Compounds **2** and **3** are new to the literature.

Compound **1**, megaphone acetate, has previously been found in *Aniba megaphylla* Mez. (Lauraceae) and showed activity against human carcinoma cells of the nasopharynx (9KB) (6). Compound **1** also showed cytotoxic activity in our panel of human tumor cells and was selectively toxic to human colon adenocarcinoma cells (HT-29). Originally, the structural elucidation of **1** was based on the ms and ^{1}H-nmr data of megaphone, the deacetylated analogue. Comparison of our ^{13}C-nmr data obtained from **1** with those of megaphone showed that the value for the C-4 carbonyl, at 201.22 ppm, was higher than that of megaphone whose value was reported at 193.37 ppm. In order to prove further the skeleton of the hexoketo-



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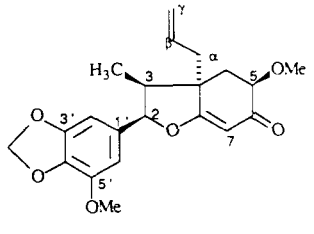

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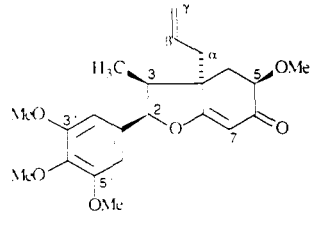

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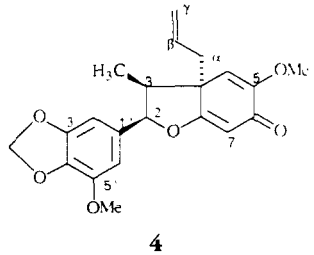

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TABLE 1. Bioactivities of Compounds **1–4**.[a]

Compound	BST LC_{50} (ppm)	PD % inhibition	A-549 ED_{50} (μg/ml)	MCF-7 ED_{50} (μg/ml)	HT-29 ED_{50} (μg/ml)
1	253.6 (549.6/137.2)[b]	48.3	2.59	3.66	5.52×10^{-1}
2	47.53 (92.41/24.43)	32.2	4.23	5.51	2.39
3	334.2 (1339.1/150.8)	52.0	3.68	4.09	2.76
4	162.1 (732.8/71.3)	inactive	>100	79.0	96.5
Adriamycin	4.05 (9.07/2.20)[c]	68.8	2.92×10^{-2}	1.15×10^{-1}	3.44×10^{-2}

[a]A-549, human lung carcinoma (11); MCF-7, human breast carcinoma; HT-29 (12), human colon adenocarcinoma (13); BST, *brine shrimp lethality test* (2); PD, *inhibition of crown gall tumors on discs of potato tubers* (3).

[b]95% confidence intervals are in parentheses.

[c]Adriamycin and other samples were treated the same way, i.e., by dissolving the compounds in organic solvent and drying aliquots in the test vials at room temperature before adding the brine shrimp. Lower LC_{50} values for adriamycin were obtained when it was dissolved in artificial sea water directly and tested at once.

ring, HETCOR and long range HETCOR analyses of **1** were performed. In addition to all of the expected correlations, the long range coupling between the carbonyl at C-4 and the double bond proton at H-6 was observed and con-

TABLE 2. ^{13}C-nmr Data of Compounds **1–4** (125 MHz, $CDCl_3$).

Carbon	Compound				
	1		**2**	**3**	**4**
	δ ^{13}C	^{13}C-^{1}H[a]			
C-1	75.69	6.52 (H-2′)			
C-2	41.04		87.18	87.34	87.19
C-3	52.19		42.58	42.61	43.93
C-3a			50.15	50.23	53.93
C-4	201.22	6.89 (H-6)	32.03	32.14	108.99
C-5	137.27		76.83	76.69	152.79
C-6	147.78		196.58	196.68	182.56
C-7	73.18	3.43 (-OMe)	100.34	100.47	102.34
C-7a			183.24	183.33	181.25
C-8	38.76				
C-α	34.50		39.02	39.13	44.63
C-β	128.91		132.55	132.62	131.64
C-γ	118.28		119.93	119.99	120.21
C-1′	135.13	5.64 (H-1)	134.72	131.69	134.75
C-2′	102.46	5.64 (H-1)	99.81	102.41	99.77
C-3′	152.51	3.79 (-OMe)	149.00	153.39	149.02
C-4′	140.52	6.52 (H-2′, H-6′)	130.51	137.47	131.11
C-5′	152.51	3.79 (-OMe)	143.56	153.39	143.58
C-6′	102.46	5.64 (H-1)	104.99	102.41	104.98
R-OMe	60.71		58.78	58.96	55.32
3′,5′-OMe	56.05		56.73	56.23	56.74
4′-OMe	56.35			60.91	
OCOMe	169.63	2.12 (-OAc)			
OCO*Me*	21.29				
OCH_2O-			101.57		101.59
3-Me	5.95		11.53	11.63	12.02

[a]Long-range correlations.

firmed that the ketone is conjugated with a double bond. Therefore, **1** was identified as megaphone acetate, and its complete ^{13}C-nmr data are given in Table 2.

High resolution mass measurements indicated molecular formulae of $C_{21}H_{24}O_6$ for **2**, $C_{22}H_{28}O_6$ for **3**, and $C_{21}H_{22}O_6$ for **4**. The diagnostic ms fragments at *m/z* 192 $[ArCHCHMe]^+$ and 179 $[ArCO]^+$ for **2** and **4** showed that both of them contained the same aromatic C_6C_3 moiety. The O-CH_2-O peak (5.9 ppm), the aromatic peak (6.4 ppm), as well as the OMe peak (3.9 ppm) in the ^{1}H nmr of **2** and **4** indicated that the aromatic moiety of both compounds is 3-methoxy-4,5-methylenedioxyphenyl. The ms fragments at *m/z* 208 $[ArCHCHMe]^+$ and 195 $[ArCO]^+$ and ^{1}H-nmr peaks at 3.87 and 3.86 ppm of compound **3** revealed that the aromatic moiety of **3** is trimethoxyphenyl. The α,β-unsaturated carbonyl peaks (ν max 1663 ± 9 cm^{-1}) in the ir spectra of **2**, **3**, and **4** suggested that these compounds belong to the benzofuranoid type of neolignans. The structure of **4** was established by ^{1}H- and ^{13}C-nmr spectra; however, compound **4** was previously isolated from an *Aniba* sp., but no bioactivity was reported (7). The molecular formula of dysodanthin A [**2**] showed two more protons than compound **4**. An ABX system could be seen in its ^{1}H-nmr spectra with protons at 4.00 ppm (J = 12.3, 5.2 Hz), 2.30 ppm (J = 12.5, 5.2 Hz), and 1.91 ppm (J = 12.5, 12.3 Hz). Double irradiation experiments suggested that this part of the structure is CH_2CHOMe. The double bond signals in the keto ring of compound **4** at 108.99 and 152.79 ppm had shifted to signals at 32.03 and 76.83 ppm in compound **2**, and the ^{13}C-nmr signal for the carbonyl group had shifted from 182.56 ppm in **4** to 196.5 ppm in **2**. These results, combined with the ^{1}H-nmr assignments (Table 3), indicated that the 4,5 double bond of the keto ring of **2** was saturated. Therefore, dysodanthin A [**2**] was recognized as a 6-oxo-hexahydrobenzofuranoid type of neolignan. Compound **3** also showed an ABX system in its ^{1}H nmr with peaks at 4.01 (J = 12.3, 5.2 Hz), 2.32 (J = 12.5, 5.2 Hz), and 1.92 ppm (J = 12.5, 12.3 Hz). With a similar carbonyl peak at 196.7 ppm in its ^{13}C nmr spectra, **3** was also identified as a 6-oxo-hexahydrobenzofuranoid type of neolignan.

There are four chiral centers in **2** and **3**. Differences in the configurations at these chiral centers can form different groups of hexahydrobenzofuranoids. The chemical shifts in the ^{1}H-nmr and ^{13}C-nmr spectra are of diagnostic importance for determination of the configuration and conformation of the hydrobenzofuranoid neolignans (7,8). The relative shielding of the 3-Me by the vicinal aromatic ring in the ^{1}H nmr (0.5 ppm) of **2** and **3** revealed the cis relationship of these groups. The 3-Me would appear at about 1.1 ppm if they were in the trans form. The chemical shifts of the methylene at C-α in the ^{1}H-nmr (2.6 ppm) and ^{13}C-nmr (39 ppm) spectra of **2** and **3** was evidence for the trans aryl/allyl relation. The axial orientation of H-5 in **2** and **3** was revealed by the coupling constants (J = 5, 12 Hz) of their ^{1}H-nmr doublets of doublets. The stereochemistries of dysodanthin A [**2**] and dysodanthin B [**3**] also were determined by comparisons of their ^{1}H- and ^{13}C-nmr spectra with those of their isomers, armenin C and canellin D, and by cd comparisons with the model compound, porosin, which has the same stereochemistry (9). Since the absolute configurations of porosin, armenin C, and canellin D were previously established (9), it is reasonable to suggest that the absolute configurations of dysodanthin A [**2**] and dysodanthin B [**3**] are as illustrated. The structures of **2** and **3**, therefore, can be described as (2*R*, 3*S*,3a*S*,5*R*)-3a-allyl-5-methoxy-2-(3-methoxy-4,5-methylenedioxyphenyl)-3-methyl-2,3,3a,4,5,6-hexahydro-6-oxo-

TABLE 3. ^{1}H-nmr Data of Compounds **1–4** (500 MHz, $CDCl_3$).

Proton	Compound δ H (*J*, Hz)			
	1	**2**	**3**	**4**
H-1	5.64 d (1.0)			
H-2	2.52 dq (1.0, 7.4)	5.81 d (5.2)	5.84 d (5.2)	5.90 d (5.1)
H-3		2.57 dq (5.2, 7.4)	2.57 dq (5.2, 7.5)	2.71 dq (5.1, 7.3)
H-4a		2.30 dd (12.5, 5.2)	2.32 dd (12.5, 5.2)	5.87 s
H-4b		1.91 dd (12.5, 12.3)	1.92 dd (12.5, 12.3)	
H-5	5.98 dd (10.3, 2.1)	4.00 dd (12.3, 5.2)	4.01 dd (12.3, 5.2)	
H-6	6.89 dt (10.3, 1.8, 1.9)			
H-7	4.19 dddd (10.1, 5.4, 2.1, 1.8)	5.57 s	5.60 s	5.50 s
H-8a	1.84 dd (13.2, 10.1)			
H-8b	2.27 ddd (13.2, 5.4, 1.9)			
H-α	2.33 d (7.3)	2.66 dd (14.3, 6.6)	2.69 dd (6.9, 15.0)	2.68 dd (13.6, 6.8)
H-α′		2.53 dd (14.3, 7.1)	2.50 dd (15.0, 7.3)	2.51 dd (13.6, 6.8)
H-β	5.55 ddt (16.8, 10.5, 7.3)	5.91 dddd (16.8, 10.1, 6.6, 7.1)	5.85 dddd (6.9, 7.3, 17.3, 11.2)	5.75 ddd (16.9, 10.1, 6.8)
H-γ	4.95 dd (16.8, 1.8)	5.31 dd (16.8, 1.4)	5.34 dd (17.0, 1.5)	5.21 dd (10.1, 1.6)
H-γ′	4.96 dd (10.5, 1.8)	5.33 dd (10.1, 1.4)	5.35 dd (11.2, 1.5)	5.14 dd (16.9, 1.6)
H-2′	6.52 s	6.40 d (1.4)	6.42 s	6.40 s
H-6′	6.52 s	6.41 d (1.4)	6.42 s	6.40 s
R-OMe	3.42 s	3.60 s	3.61 s	3.69 s
3′,5′-OMe	3.86 s	3.92 s	3.87 s	3.93 s
4′-OMe	3.79 s		3.86 s	
OAc	2.12 s			
OCH_2O-		5.99 s		5.99 s
3-Me	0.90 d (7.3)	0.53 d (7.4)	0.54 d (7.5)	0.52 d (7.3)

benzofuran [**2**], and (2*R*,3*S*,3a*S*,5*R*)-3a-allyl-5-methoxy-2-(3,4,5-trimethoxyphenyl)-3-methyl-6-oxo-2,3,3a,4,5,6-hexahydrobenzofuran [**3**], respectively. Both **2** and **3** are new compounds. Also, the stereochemistries of compounds **1** and **4** were determined as illustrated by comparisons of their ^{1}H-nmr, ^{13}C-nmr, and cd data with those that were reported (6,10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were determined on a Varian VXR-500S spectrometer. Low resolution cims and eims were recorded on a Finnigan 4000. Hrms spectra were determined on a Kratos MS-50. Ir spectra were obtained on a Perkin-Elmer 1600 series FTIR. Cd spectra were measured in MeOH using the JASCO Model J600 Circular Dichroism Spectropolarimeter. Mp's were measured in capillaries on a Mel-temp apparatus and were uncorrected.

PLANT MATERIAL.—The roots of *E. dysodantha* were collected in Peru under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, Maryland, where voucher specimens (B811608, BRS-10-191) are maintained.

EXTRACTION AND ISOLATION.—The whole extraction and isolation procedure was directed by the brine shrimp lethality test. The powdered roots (2 kg) were extracted with 95% EtOH. The extract residue (F001) was then partitioned between CH_2Cl_2 and H_2O. The residue of the CH_2Cl_2 fraction (F003) was subjected to column and Chromatotron chromatographic separations eluted with hexane, CH_2Cl_2, and MeOH in various gradients. The neolignans were isolated in the medium polar fractions.

MEGAPHONE ACETATE [**1**].—Compound **1** (158 mg, 0.0079% yield): oil $[\alpha]_D$ −9.2° (EtOH, c = 0.046); uv λ max (MeOH) 240 (ε 12652), 265 (ε 1957); ir ν max (film) 2936, 1748, 1716, 1676, 1654, 1592, 1558, 1541, 1508, 1458, 1420, 1385, 1338, 1132, 1109, 1014, 919; exact mass found *m/z* 432.2146 (calcd for $C_{24}H_{32}O_7$ $[M]^+$ 432.2148); eims *m/z* (% rel. int.) $[M]^+$ 432 (35), 266 (72), 224 (65), 197 (100), 169 (28); ^{1}H nmr see Table 3; ^{13}C nmr see Table 2.

DYSODANTHIN A [**2**].—Compound **2** (125 mg, 0.0063% yield): colorless needles; mp 172° (crystallized from CH_2Cl_2 and Et_2O mixture); cd [θ] max 305 (+24101), 264 (−7291), 250 (−2791); ir ν max (film) 2967, 2824, 1654, 1634, 1508, 1450, 1431, 1324, 1179, 1127, 1092, 1043, 934, 837, 802; eims *m/z* (% rel. int.) $[M+1]^+$ 373 (21), $[M]^+$ 372 (8), 342 (18), 205 (37), 192 (19), 179 (14), 165 (26); hrms *m/z* $[M]^+$ 372.1565 (calcd 372.1573 for $C_{21}H_{24}O_6$); ^{1}H nmr see Table 3; ^{13}C nmr see Table 2.

DYSODANTHIN B [**3**].—Compound **3** (8 mg, 0.0004% yield): colorless oil; cd [θ] max 303 (+13858), 264 (−26624), 252 (−25425); ir ν max (film) 2918, 2839, 1658, 1629, 1589, 1506, 1457, 1354, 1235, 1177, 1123, 936, 832, 725; eims *m/z* (% rel. int.) $[M+1]^+$ 389 (31), $[M]^+$ 388 (40), 358 (42), 221 (66), 208 (24), 195 (18), 181 (43); hrms *m/z* $[M+1]^+$ 389.1952 (calcd 389.1964 for $C_{22}H_{29}O_6$); ^{1}H nmr see Table 3; ^{13}C nmr see Table 2.

(2*R*,3*S*,3a*S*)-3a-ALLYL-5-METHOXY-2-(3-METHOXY-4,5-METHYLENEDIOXYPHENYL)-3-METHYL-2,3,3a,6-TETRAHYDRO-6-OXOBENZOFURAN [**4**].—Compound **4** (92 mg, 0.0046% yield): colorless plates; mp 182° (crystallized from CH_2Cl_2 and Et_2O mixture); cd [θ] max 330 (+9300), 266 (−7849), 252 (−4106); ir ν max 2965, 2822, 1654, 1611, 1510, 1143, 1386, 1237, 1161, 1089, 1041, 932, 845; eims *m/z* (% rel. int.) $[M]^+$ 370 (19), 329 (11), 205 (5), 192 (14), 165 (18); hrms *m/z* $[M]^+$ 370.1419 (calcd 370.1416 for $C_{21}H_{22}O_6$); ^{1}H nmr see Table 3; ^{13}C nmr see Table 2.

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