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RAMOSISSIN AND OTHER METHOXYLATED NOR-NEOLIGNANS FROM *KRAMERIA RAMOSISSIMA**

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Key Word Index—*Krameria ramosissima*; Krameriaceae; nor-neolignans; ramosissin; 2-(2,3,4,5,6-pentamethoxyphenyl)-5-(*E*)-propenylbenzofuran.

Abstract—From the hexane extract of the roots of *Krameria ramosissima* six new nor-neolignans were isolated, among them the highly methoxylated compounds 2-(2,3,4,5,6-pentamethoxyphenyl)-5-(*E*)-propenylbenzofuran (ramosissin) and 2-(2,3,4,6-tetramethoxyphenyl)-5-(*E*)-propenylbenzofuran. Structure elucidation was based mainly on spectroscopic investigations, particularly on the ^{13}C NMR spectra.

INTRODUCTION

Krameria ramosissima Gray, common Spanish name 'calderona', is a small, highly branched shrub native in the northern Mexican arid zones. In some regions its boiled roots are used to prepare a tea against diarrhoea and mild fevers.

From a chemotaxonomical point of view the occurrence of related lignans, neolignans and nor-neolignans in various *Krameria* species [1–3] corroborates the taxonomic separation of the Krameriaceae family from the Leguminosae [4–6].

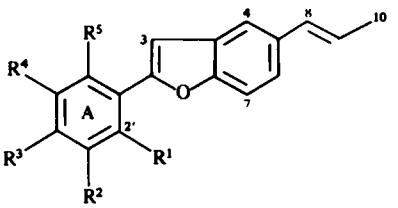
RESULTS

Repeated chromatography of the hexane extract yielded the known compounds ratanhiaphenol I (**1**) [1–3] and hermosillol (**8**) [1], and the new nor-neolignans **2**–**7**.

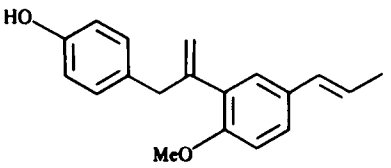
The ^1H NMR spectrum of the main compound **2** showed a structural relationship to **1**. However, in the aromatic range there were signals of four protons only and these could be assigned to a 2-substituted 5-propenylbenzofuran [7, 8]. Signals of five partly equivalent methoxy groups and the mass spectrum were consistent with the 2-pentamethoxyphenyl-substituted formula **2**.

Additional proof of the structure was provided by the ^{13}C NMR spectrum. ^{13}C resonances of methoxy substituents on a benzene ring appear at 55–57 ppm, if there is at least one *ortho* position unsubstituted, whereas resonance values at 59–63 ppm are typical if both *ortho* positions are substituted [9–12]. The ^{13}C NMR spectrum of **2** exhibits three signals for five methoxy groups (because of symmetry) all at $\delta > 61.5$ (Table 1) and this demands a pentamethoxylated ring A in **2**. Carbon atoms 9 and 10 were removed by oxidation with $KMnO_4$ and this yielded the corresponding benzoic acid.

The structures of the closely related compounds **3**–**7** were also established by spectroscopic investigations. In particular ^{13}C NMR experiments served to place the substituents at ring A unambiguously.



	R¹	R²	R³	R⁴	R⁵
1	OH	H	OMe	H	H
2	OMe	OMe	OMe	OMe	OMe
3	OMe	OMe	OMe	H	OMe
4	OMe	H	OMe	H	OMe
5	OH	H	OMe	H	OMe
6	OH	H	OMe	H	OH
7	OMe	H	OMe	H	H



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Ratanhiaphenol I (**1**) is a compound widely distributed in the Krameriaceae. For the first time **1** has been described as a major component of *K. triandra* [2] and it is present among the constituents of *K. cystisoides* [3] and *K. sonorae* [1]. Hermosillol (**8**) had only previously been isolated from *K. sonorae* [1]. Ramosissin (**2**) is the first compound in the lignan and neolignan series, respectively, carrying a pentamethoxyphenyl moiety.

* Part 3 in the series "Studies on Krameriaceae". For part 2 see ref. [1].

Table 1. ^{13}C NMR resonances of compounds 2–7 (in $CDCl_3$, unless otherwise stated)

	2	2*	3	4	5	6*	7
C-2	154.17	155.05	154.17†	154.14	156.72	153.56†	153.20†
C-3	107.34	107.97	107.20	107.15	105.63	106.67	105.14‡
C-3a	129.12	130.19	129.31	129.39	128.98	130.24	130.36
C-4	117.90	118.89	117.82	117.71	117.77	118.48	117.87
C-5	132.99	134.03	132.88‡	132.67†	133.77	134.28	133.02
C-6	122.13	123.12	121.88	121.59	122.10	122.66	121.94
C-7	110.94	111.60	110.94	110.89	110.32	111.47	110.48
C-7a	149.76	151.01	150.24	150.60	151.87	152.91†	152.95†
C-8	131.28	132.27	131.47‡	131.55†	131.12	132.22	131.50
C-9	124.19	124.77	124.02	123.78	124.59	124.79	123.97
C-10	18.30	18.56	18.36	18.36	18.30	18.48	18.33
C-1′	114.71	115.56	107.71	102.73	99.32	99.74	113.05
C-2′	148.29	149.28	153.47†	160.10	156.72	158.06	157.91
C-3′	143.45	144.49	137.03	91.33	94.42	95.13	98.99
C-4′	148.65	149.99	154.71†	162.32	161.73	162.69	161.05
C-5′	143.45	144.49	93.44	91.33	91.90	95.13	104.39‡
C-6′	148.29	149.28	154.71†	160.10	158.75	158.06	128.03
CH_3O	61.24	61.52	56.25	55.39	55.33	55.53	55.44
	61.34	61.65	56.55	56.12	55.71		55.52
		61.76	61.10				
			61.43				

*In acetone-d_6.

††Assignments may be interchanged.

The close biogenetic relationship of compounds 1–7 is obvious.

EXPERIMENTAL

Plant material. Plants were collected around Marin, Nuevo León (Mexico) in May 1985; a voucher specimen is kept at our herbarium (Reg. no. 7932) in Monterrey.

Mps are uncorr. TLC was performed on ready made plates (Nano plates Sil-20 UV, Macherey-Nagel) using the solvent systems: S-1, cyclohexane–EtOAc (4:1); S-2, $CHCl_3$–MeOH (24:1); S-3, CH_2Cl_2–$CHCl_3$ (9:1); S-4, CH_2Cl_2; detection by UV and anisaldehyde [13] followed by heating. IR spectra were recorded in $CHCl_3$ solns and KBr pellets, respectively. UV were recorded in MeOH. ^1H NMR and ^{13}C NMR spectra were recorded at 90 and 22.50 MHz, respectively, in $CDCl_3$, unless otherwise stated; internal standard TMS. MS were determined at 70 eV using direct inlet system.

Extraction and chromatography. Roots (960 g) were extracted successively with hexane and MeOH to yield 6.4 g hexane extract and 150 g MeOH extract. The hexane extract was repeatedly chromatographed on silica gel using cyclohexane–EtOAc, $CHCl_3$–CH_2Cl_2 and CH_2Cl_2 (the latter at 7°). This yielded compounds 1–8.

Identification of ratanhiaphenol I (1) *and hermosillol* (8). The identification is based on comparison with authentic substances. Compound 1 was obtained from *Krameria cystisoides* [3] and 8 from *K. sonorae* [1].

2-(2,3,4,5,6-*Pentamethoxyphenyl*)-5-(E)-*propenylbenzofuran* (2). Colourless crystals (130 mg): mp 92–94°; R_f (S-1) 0.44, green with anisaldehyde; IR ν_{max}^{KBr} cm^{-1}: 2930, 1470; UV λ_{max} nm (log ε): 233 (4.55), 255 (sh, 4.51), 270 (sh, 4.45), 285 (sh, 4.39), 317 (sh, 4.40); + NaOH: unchanged; ^1H NMR (acetone-d_6): δ 1.87 (3H, *br d*, J = 6 Hz, Me-10), 3.75 (6H, *s*, MeO-2′,6′), 3.88 (6H, *s*, MeO-3′,5′), 3.97 (3H, *s*, MeO-4′), 6.27 (1H, *dq*, J_1 = 16, J_2 = 6 Hz, H-9), 6.52 (1H, *br d*, J = 16 Hz, H-8), 6.93 (1H, *d*, J = 1 Hz, H-3), 7.34 (1H, *dd*, J_1 = 8.5, J_2 = 1.5 Hz, H-6), 7.46 (1H, *ddd*, J_1 = 8.5, $J_2 = J_3$ = 1 Hz, H-7), 7.59 (1H, *br s*, H-4); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 384 (100, M^+), 369 (5), 341 (4), 327 (4), 326 (19), 311 (4), 283 (9), 255 (3), 192 (4), 163 (4).

2-(2,3,4,5,6-*Pentamethoxyphenyl*)-5-*benzofurancarboxylic acid by oxidation of* 2. A soln of $KMnO_4$ in Me_2CO (10%) was added dropwise to a soln of 6 mg 2 in Me_2CO (3 ml) until the colour remained. After stirring for 20 hr (room temp.) the solvent was evapd and 10% KOH (10 ml) was added. The ppt was filtered off. Then the filtrate was extracted with *tert*-butyl methyl ether, acidified with conc. HCl and extracted with $CHCl_3$. The residue of the $CHCl_3$ extract was purified on silica gel using S-2 to yield 2.4 mg oily product: R_f (S-2) 0.11, grey with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 2940, 1710, 1685, 1610; ^1H NMR (acetone-d_6): δ 3.79 (6H, *s*, 2 × MeO), 3.89 (6H, *s*, 2 × MeO), 3.99 (3H, *s*, MeO), 7.11 (1H, *d*, J = 1 Hz, H-3), 7.64 (1H, *ddd*, J_1 = 9, $J_2 = J_3$ = 1 Hz, H-7), 8.06 (1H, *dd*, J_1 = 9, J_2 = 1.5 Hz, H-6), 8.43 (1H, *dd*, J_1 = 1.5, J_2 = 1 Hz, H-4); MS *m/z* (rel. int.): 388.1159 ($C_{20}H_{20}O_8$; 100, M^+), 373 (13), 345 (17), 331 (8), 330.0738 ($C_{17}H_{14}O_7$; 44), 315 (5), 287 (30), 259 (16), 213 (5), 201 (13), 194 (8), 186 (4), 165 (26).

2-(2,3,4,5,6-*Pentamethoxyphenyl*)-5-*benzofurancarboxylic acid methyl ester.* A soln of CH_2N_2 in Et_2O was added dropwise to 1.5 mg carboxylic acid in MeOH (5 ml) until the yellow colour remained. After 3 hr the solvent was evapd to yield 1.5 mg oily product: R_f (S-2) 0.80, grey with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 1710 (C=O), 1610; ^1H NMR (acetone-d_6): δ 3.78 (6H, *s*, 2 × MeO), 3.88 (6H, *s*, 2 × MeO), 3.93 (3H, *s*, CH_3–CO), 3.99 (3H, *s*, MeO), 7.12 (1H, *d*, J = 1 Hz, H-3), 7.66 (1H, *ddd*, J_1 = 9, $J_2 = J_3$ = 1 Hz, H-7), 8.04 (1H, *dd*, J_1 = 9, J_2 = 1.5 Hz, H-6), 8.43 (1H, *dd*, J_1 = 1.5, J_2 = 1 Hz, H-4); MS *m/z* (rel. int.): 402 (100, M^+), 387 (9), 371 (6), 359 (11), 344 (38), 329 (5), 301 (30), 273 (15), 215 (14), 201 (12), 186 (16), 178 (15), 171 (6), 164 (7), 157 (25), 135 (25), 121 (13).

2-(2,3,4,6-*Tetramethoxyphenyl*)-5-(E)-*propenylbenzofuran* (3). Colourless crystals (32 mg): mp 125–127°; R_f (S-3) 0.29,

grey with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2940, 2920, 2840, 1610, 1585; UV λ_{max} nm (log ε): 234 (4.64), 258 (sh, 4.58), 292 (sh, 4.56), 320 (sh, 4.37); ^{1}H NMR: δ1.89 (3H, *br d*, J = 6 Hz, Me-10), 3.80 (6H, *s*, 2 × MeO), 3.85 (3H, *s*, MeO), 3.92 (3H, *s*, MeO), 5.91–6.31 (1H, *m*, H-9), 6.35 (1H, *s*, H-5'), 6.55 (1H, *br d*, J = 16 Hz, H-8), 6.82 (1H, *br s*, H-3), 7.24 (1H, *dd*, J_1 = 8, J_2 = 1.5 Hz, H-6), 7.40 (1H, *br d*, J = 8 Hz, H-7), 7.51 (1H, *br s*, H-4); ^{13}C NMR see Table 1; MS *m/z* (rel. int.): 354 (100, M$^+$), 339 (12), 296 (10), 281 (5), 225 (6), 177 (4), 170 (4), 148 (4).

2-(2,4,6-*Trimethoxyphenyl*)-5-(E)-*propenylbenzofuran* (**4**). Colourless crystals (31 mg); mp 139–140°; R_f (S-1) 0.30, grey-blue with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2940, 2920, 2840, 1615, 1585; UV λ_{max} nm (log ε): 232 (4.55), 255 (sh, 4.52), 320 (sh, 4.23); ^{1}H NMR: δ1.89 (3H, *br d*, J = 6 Hz, Me-10), 3.77 (6H, *s*, 2 × MeO), 3.84 (3H, *s*, MeO), 5.97–6.35 (1H, *m*, H-9), 6.19 (2H, *s*, H-3',H-5'), 6.49 (1H, *br d*, J = 16 Hz, H-8), 6.74 (1H, *d*, J = 1 Hz, H-3), 7.26 (1H, *dd*, J_1 = 8.5, J_2 = 1.5 Hz, H-6), 7.42 (1H, *br d*, J = 8.5 Hz, H-7), 7.48 (1H, *br s*, H-4); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 324 (100, M$^+$), 309 (3), 281 (6), 266 (5), 178 (16), 162 (5).

2-(4, 6-*Dimethoxy-2-hydroxyphenyl*)-5-(E)-*propenylbenzofuran* (**5**). Colourless crystals (20 mg); mp 119–120°; R_f (S-4) 0.58, yellow-brown with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480 (OH), 2960, 2940, 2920, 2840, 1620, 1585; UV λ_{max} nm (log ε): 233 (4.68), 246 (sh, 4.65), 256 (sh, 4.63), 294 (4.49), 307 (sh, 4.47), 320 (sh, 4.45), 332 (sh, 4.33); + NaOH: 239 (4.72), 321 (4.38), 332 (sh, 4.37); ^{1}H NMR (acetone-d_6): δ1.87 (3H, *br d*, J = 6 Hz, Me-10), 3.82 (3H, *s*, MeO), 3.85 (3H, *s*, MeO), 6.24 (2H, *br s*, H-3', H-5'), 6.08–6.40 (1H, *m*, H-9), 6.53 (1H, *br d*, J = 16 Hz, H-8), 6.95 (1H, *br s*, H-3), 7.28 (1H, *dd*, J_1 = 9, J_2 = 1.5 Hz, H-6), 7.45 (1H, *br d*, J = 9 Hz, H-7), 7.56 (1H, *br s*, H-4), 8.50 (1H, *s*, OH); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 310 (100, M$^+$), 295 (15), 164 (5), 155 (5).

2-(2,6-*Dihydroxy-4-methoxyphenyl*)-5-(E)-*propenylbenzofuran* (**6**). Colourless crystals (52 mg); mp 167–168°; R_f (S-3) 0.25, yellow-brown with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 3500 (OH), 2960, 2940, 2920, 2830, 1635, 1590; UV λ_{max} nm (log ε): 236 (4.56), 247 (sh, 4.55), 258 (sh, 4.52), 293 (4.47), 305 (4.47), 318 (4.45), 326 (sh, 4.42), 332 (sh, 4.39); + NaOH: 243 (4.59), 277 (sh, 4.37), 305 (4.39), 336 (4.42); ^{1}H NMR (acetone-d_6): δ1.84 (3H, *br d*, J = 5 Hz, Me-10), 3.76 (3H, *s*, MeO), 6.18 (2H, *s*, H-3', H-5'), 5.99–6.36 (1H, *m*, H-9), 6.49 (1H, *br d*, J = 16 Hz, H-8), 7.13 (1H, *d*, J = 1 Hz, H-3), 7.27 (1H, *dd*, J_1 = 8, J_2 = 1.5 Hz, H-6), 7.44 (1H, *br d*, J = 8 Hz, H-7), 7.54 (1H, *br s*, H-4), 8.72 (2H, *br s*, 2 OH); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 296 (100, M$^+$), 281 (30).

2-(2,4-*Dimethoxyphenyl*)-5-(E)-*propenylbenzofuran* (**7**). Colourless crystals (7 mg); mp 146–147°; R_f (S-4) 0.70, grey with anisaldehyde; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2940, 2920, 2840, 1610, 1585; UV λ_{max} nm (log ε): 232 (4.61), 255 (sh, 4.55), 320 (sh, 4.26); ^{1}H NMR: δ1.88 (3H, *br d*, J = 5.5 Hz, Me-10), 3.83 (3H, *s*, MeO), 3.94 (3H, *s*, MeO), 6.16 (1H, *dq*, J_1 = 16, J_2 = 5.5 Hz, H-9), 6.47 (1H, *br d*, J = 16 Hz, H-8), 6.54–6.69 (2H, *m*, H-3', H-5'), 7.13 (1H, *br s*, H-3), 7.22 (1H, *dd*, J_1 = 8, J_2 = 1.5 Hz, H-6), 7.37 (1H, *br d*, J = 8 Hz, H-7), 7.46 (1H, *br s*, H-4), 7.94 (1H, *br d*, J = 9 Hz, H-6'); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 294 (100, M$^+$), 279 (10), 251 (6), 236 (3), 165 (3), 148 (9), 147 (3).

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