The Peltatols, Novel HIV-Inhibitory Catechol Derivatives from *Pothomorphe peltata*[1]

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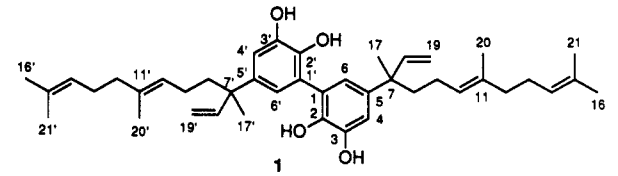
Three new prenylated catechol dimers have been isolated from the tropical shrub *Pothomorphe peltata* (Piperaceae). The structures of peltatol A (1), peltatol B (2), and peltatol C (3) were determined by extensive spectroscopic analyses. Peltatols A–C inhibited HIV-1 induced cell killing at subcytotoxic concentrations of 1–10 μg/mL. The monomeric catechol derivative 4-nerolidylcatechol (4) was active in a phorbol receptor binding assay (IC_{50} = 35 μg/mL) but inactive against HIV. The diphenyl ethers, peltatols B (2) and C (3), were interconvertible in solution (MeOH, DMSO) at room temperature.

The National Cancer Institute has initiated a major new screening program to isolate and identify natural products with anti-HIV[2] or antitumor properties. Extracts from a diverse range of terrestrial plants, marine organisms, and microbial sources are being tested for anti-HIV activity or selective cytotoxic properties. As part of this effort, an NCI contract collection of the tropical shrub *Pothomorphe peltata* (L.) Miq. (Piperaceae), synonomous with *Lepianthes peltatum* (L.) Raf., was made in the Dominican Republic. The CH_2Cl_2/MeOH extract of dried, finely milled roots was found to have activity in the anti-HIV screen.[3] This extract was additionally active in a phorbol dibutyrate (PDBu) receptor binding assay,[4] which detects compounds which bind at or near the phorbol recognition site. While PDBu receptor binding activity is generally associated with phorbol diterpenes, a number of other structural chemotypes have been found active in this assay. Interestingly, we have observed that compounds active in the PDBu assay often inhibit HIV-1 induced cell killing in vitro. Examples of PDBu active compounds which also block the cytopathic effects of HIV-1 include phorbol ester diterpenes,[5] indolactams,[6] and aplysiatoxins.[6]

A bioassay-directed separation of the *P. peltata* extract tracked activity using both the anti-HIV and PDBu assays. Sequential application of solvent partitioning, gel permeation on Sephadex LH-20, and reversed-phase C_{18} HPLC led to one HIV-inhibitory fraction containing peltatols A, B, and C (1–3) and a PDBu active fraction consisting of 4-nerolidylcatechol (4). Compounds 1–3 are novel structures while catechol derivative 4 has previously been isolated from *P. umbellata*.[7]

Peltatol A (1) was isolated as an optically active, pale yellow oil. A broad IR absorption at 3370 cm^{-1} and bathochromic shifts in the UV spectrum upon addition of base were indicative of phenolic functionalities. The molecular ion by HREIMS at *m/z* 626.4335 Da corresponded to a molecular formula of $C_{42}H_{58}O_4$. The ^{13}C NMR spectrum of 1, however, contained only 21 resolved carbon resonances and the total apparent integration in the ^1H NMR spectrum was for 29 protons; this could best be explained if 1 was a symmetrical dimer and the NMR resonances were all doubled. The ^1H and ^{13}C NMR spectra contained signals that were virtually identical to those



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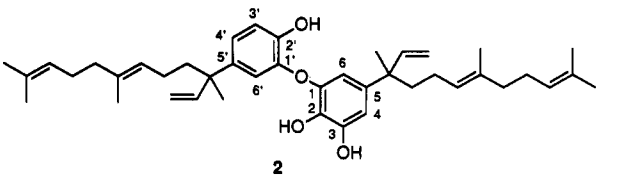

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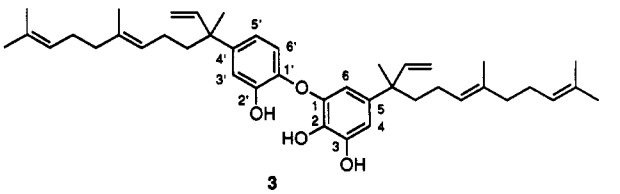

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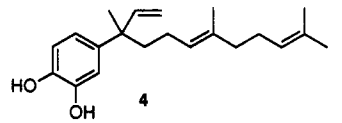

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reported for the aliphatic portion of 4.[7] Characteristic ^1H resonances, including two resolved protons of a vinyl group (δ 5.05 dd, *J* = 17.5, 1.5 Hz; 6.00 dd, *J* = 17.5, 10.8 Hz), three olefinic methyls (δ 1.51, 1.57, 1.65), and a methyl singlet (δ 1.35), indicated that each half of the molecule

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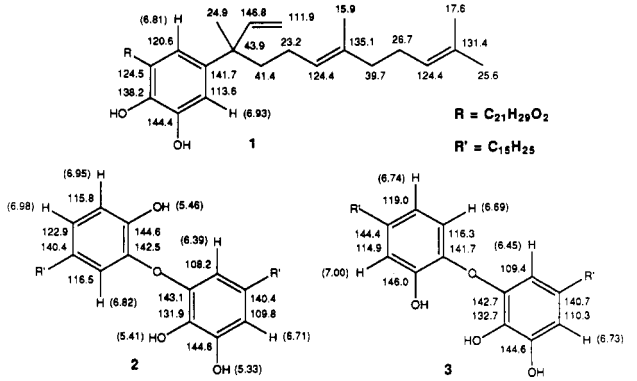

Figure 1. Selected ^1H and ^{13}C NMR assignments for peltatol A (1), peltatol B (2) and peltatol C (3).

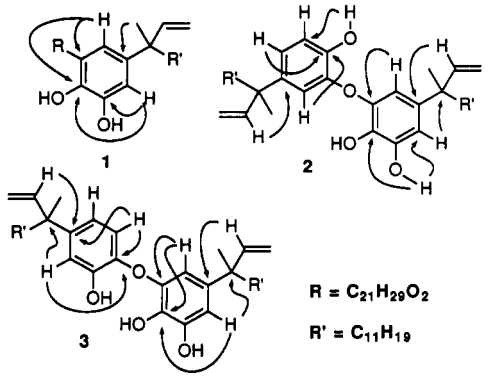

Figure 2. Selected HMBC correlations of peltatol A (1), peltatol B (2), and peltatol C (3).

contained a $C_{15}H_{25}$ nerolidyl substituent. Peltatol A (1) showed two exchangeable phenolic proton signals (δ 5.74, 6.21) and only two aromatic proton doublets (δ 6.81, 6.93) that were meta coupled (J = 2.3 Hz). This revealed that the two halves of the molecule were joined via a biphenyl linkage. Proton-detected heteronuclear correlation experiments (HMQC, HMBC) allowed assignment of the ^1H and ^{13}C NMR resonances (Figure 1). The C4 (δ 113.6) and C6 (δ 120.6) protonated carbons were defined by one-bond C–H correlations, while C5 (δ 141.7) was assigned by an HMBC correlation (Figure 2) with the C17 methyl group (δ 1.35). Both aromatic protons correlated to the C2 phenolic carbon (δ 138.2) and to C7 (δ 43.9) of the aliphatic side chain. The resonance at δ 6.93 (H4) also correlated to the C3 (δ 144.4) phenol bearing carbon. A two bond correlation observed between H6 (δ 6.81) and C1 (δ 124.5) established that C1 was the point of attachment between the subunits. Thus, peltatol A (1) consisted of two symmetrically substituted catechols joined at C1 by a biphenyl linkage.

Peltatol B (2), isolated as a colorless oil, also analyzed for $C_{42}H_{58}O_4$ by HREIMS, with a molecular ion at m/z 626.4340 Da. A broad IR band centered at 3415 cm^{-1} and the UV (MeOH) absorbance λ_{max} 281 nm (ϵ 4500) were appropriate for nonconjugated catechol moieties. Analysis of the ^1H and ^{13}C NMR features of 2 indicated that it was closely related to peltatol A (1). However, peltatol B (2) was clearly not symmetrical, as the ^{13}C NMR spectrum contained 26 resolved carbon signals, with 16 of these resonances due to two carbons each. Characteristic ^1H and ^{13}C resonances supported the presence in 2 of two nerolidyl substituents. The remaining $C_{12}H_8O_4$ aromatic portion of the molecule provided a three-proton spin system (δ 6.82 d, J = 2.0 Hz; 6.95 d, J = 8.5 Hz; 6.98 dd, J = 8.5, 2.0 Hz) similar to that observed in the ^1H NMR of compound 4 and a meta-coupled (J = 2.0 Hz) pair of doublets (δ 6.39, 6.71) similar to those in 1. Peltatol B (2) apparently differed from peltatol A (1) in the linkage of the catechol groups. In contrast to compound 1, the ^1H NMR spectrum of 2 showed only three exchangable phenol proton signals (δ 5.33, 5.41, 5.46). These data suggested that the subunits of 2 were joined by a diphenyl ether bridge that incorporated one of the catechol oxygens. The substitution and NMR assignments (Figure 1) of both catechol groups were established with the aid of heteronuclear correlation data (Figure 2).

In the disubstituted catechol ring of 2, both aromatic protons correlated with C7 of the side chain, establishing that they were ortho to the nerolidyl substituent. The C5 carbon at δ 140.4 was identified as the point of attachment of the nerolidyl side chain by a correlation with the H17 vinyl proton. Both aromatic protons also showed three-bond HMBC correlations to the C2 phenolic carbon at δ 131.9. Correlations from a phenol proton resonance at δ 5.33 to C2 and C4 (δ 109.8) positioned a phenol substituent on C3. These HMBC results required the δ 143.1 carbon (C1) to be substituted with the oxygen of the diphenyl ether link and adjacent to both C2 and the protonated C6 carbon (δ 108.2).

Heteronuclear correlation experiments (Figure 2) established that the remaining monosubstituted catechol moiety was similar to that observed in compound 4. The question of which phenolic group was part of the ether link was solved by multiple bond HMBC data. Key correlations included those between C2′ (δ 144.6) and the two meta-coupled protons (δ 6.82, 6.98) and between the C2′ phenolic proton (δ 5.46) and C3′ (δ 115.8). This confirmed that the oxygen meta to the nerolidyl group comprised the ether link of the molecule and that peltatol B is consistent with structure 2.

Peltatol C (3), isolated as a colorless oil, analyzed for $C_{42}H_{58}O_4$ by HREIMS with a molecular ion at m/z 626.4335 Da. The IR and UV data of 3 showed close correspondence to the values recorded for 2. The ^{13}C NMR spectrum revealed the nonsymmetrical nature of peltatol C (3) as it contained 25 individual resonances, seven signals corresponding to two carbons each, and one signal for three overlapping carbons. Resonances in the ^1H and ^{13}C spectra of 3 clearly defined two linked catechols, each substituted with a nerolidyl group. Peltatol C appeared to be a positional isomer of 2 in which the oxygen para to the nerolidyl group on the monosubstituted catechol was incorporated into the diphenyl ether bridge. Heteronuclear correlation experiments (Figure 2) supported this assignment and confirmed that the ether oxygen attachment to the disubstituted catechol in 3 was meta to its nerolidyl group, similar to the substitution pattern observed in 2.

In a rather surprising finding, peltatols B (2) and C (3) were observed to interconvert cleanly in solutions of MeOH or DMSO. A pure sample of peltatol B (2) at 25 °C in deuterated MeOH was monitored by ^1H NMR as it gradually interconverted to an approximately 1:1 mixture of 2 and 3 over the course of 24 h. No other products or signs of decomposition were observed, and the resulting mixture was stable at room temperature for at least 48 h. In a similar fashion, peltatol C, in deuterated MeOH, provided a 1:1 mixture of 2 and 3 within 24 h. The interconversion of both compounds also occurred in DMSO, but a 1:1 equilibrium mixture was obtained in approximately 4 h. No interconversions of either compound were detected in $CDCl_3$, $CHCl_3$, or CH_2Cl_2 after 48 h. No precedent has been found in the literature for this type of intramolecular rearrangement of diphenyl ether linked phenolic compounds.

The metabolite 4-nerolidylcatechol (4) was an abundant constituent of the organic extract, making up approximately 24% of the extract mass. Its structure was deduced by spectroscopic analysis and confirmed by comparison of its spectral features with published values.[7] Compound 4 is optically active; we observed $\alpha_D = +3.3°$ (lit.[7] = +1.0°). The stereochemistry of the lone chiral center in 4 has never been resolved. In an attempt to establish the stereochemistry in this series of catechols, several heavy atom derivatives of the monomer 4 and the symmetrical dimer 1 were prepared. Crystalline (*p*-bromophenyl)urethane derivatives of both 1 and 4 were obtained, but they failed to provide crystals suitable for X-ray analysis.

Peltatol A (1) inhibited the cytopathic effects of HIV-1 infection in a human T-lymphocytic (CEM-SS) cell line. Cellular viability was assessed by the XTT tetrazolium assay employed in the National Cancer Institute's primary anti-HIV screen.[3] Confirmatory assays[8] measured production of viral p24 antigen and reverse transcriptase activity. Peltatol A (1) was cytoprotective over a concentration range of 1–10 μg/mL (maximum protection = 75%) and cytotoxic at ≥20 μg/mL. The dose-dependent increase in cellular viability observed in the XTT assay correlated well with concommitant reductions in viral p24 and reverse transcriptase production. A 1:1 mixture of peltatol B (2) and peltatol C (3) showed a similar activity profile against HIV infection. The monomeric catechol derivative 4-nerolidylcatechol (4) was inactive in the anti-HIV screen;[3] however, it did weakly block phorbol receptor binding[4] with an IC_{50} of 35 μg/mL.

Experimental Section

General. NMR spectra were recorded on a 500-MHz spectrometer using a sample concentration of 10 mg/mL and $CDCl_3$ as solvent and internal standard. The number of attached protons for the ^{13}C NMR signals were determined from DEPT experiments. Proton detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 140 Hz) and HMBC (optimized for $^nJ_{HC}$ = 8.2 Hz) pulse sequences.

Collection and Extraction. Samples of the tropical shrub *Pothomorphe peltata* were collected under NCI contract by Dr. T. Zanoni in the Dominican Republic. A voucher specimen for botanical reference is maintained at the Smithsonian Institution. Fresh plant material was air dried and then stored at −20 °C until processed. The roots of *P. peltata* were ground in a Wiley mill to a free flowing powder (393 g) and successively extracted with 1:1 MeOH/CH_2Cl_2 and 100% MeOH. Removal of the solvent under reduced pressure gave 38.6 g of crude extract.

Chromatographic Separation. A 17.3-g portion of the extract was partitioned between $CHCl_3$ (3 × 350 mL) and 80% aqueous methanol (300 mL). The combined $CHCl_3$ layers were evaporated in vacuo to give 13.4 g of a viscous oil. The $CHCl_3$ soluble material was fractionated on Sephadex LH-20 (5- × 100-cm column), eluting with hexane/CH_2Cl_2/MeOH (2:5:1). Early eluting fractions consisted primarily of fats and pigments while the prenylated catechols (blue to purple charring TLC spots with vanillin–H_2SO_4 spray reagent) were concentrated in the later fractions. HPLC (Rainin Dynamax C_{18}, 2.1 × 25 cm) with MeOH/H_2O (9:1) provided the pure monomeric catechol derivative 4 (4.2 g, calculated 2.4% of the dry weight) and a mixture of the dimeric catechols. Peltatol A (1) (314 mg, calculated 0.2% of the dry weight) was obtained by C_{18} HPLC using MeOH/H_2O (19:1) while peltatol B, 2 (6 mg, calculated 0.003% of the dry weight), and peltatol C, 3 (6 mg, calculated 0.003% of the dry weight), were purified by repeated C_{18} HPLC with MeOH/H_2O (49:1).

Peltatol A (1): pale yellow oil; $[\alpha]_D$ +14.2° (CH_2Cl_2, *c* 2.3); UV λ_{max} (MeOH) 291 (ε 4400), 253 (ε 9950), 224 (ε 24300) nm; λ_{max} (MeOH/NaOH) 310 (ε 5560), 264 (shoulder), 232 (ε 23800), 218 (ε 22700) nm; IR (film) ν_{max} 3370 (broad), 2966, 2923, 2855, 1593, 1494, 1450, 998 cm^{-1}; thermal desorption HREIMS *m/z* 626.4329 (calcd for $C_{42}H_{58}O_4$, 626.4335); ^1H NMR ($CDCl_3$) δ 1.35 (s, 6 H), 1.51 (s, 6 H), 1.57 (s, 6 H), 1.65 (s, 6 H), 1.68–1.89 (m, 8 H), 1.93 (m, 4 H), 2.03 (m, 4 H), 5.05 (dd, *J* = 17.5, 1.5 Hz, 2 H), 5.06–5.11 (m, 6 H), 5.74 (br, exch, 2 H), 6.00 (dd, *J* = 17.5, 10.8 Hz, 2 H), 6.21 (br, exch, 2 H), 6.81 (d, *J* = 2.3 Hz, 2 H), 6.93 (d, *J* = 2.3 Hz, 2 H); ^{13}C NMR ($CDCl_3$, all signals 2 C) δ 15.9 (3 H), 17.6 (3 H), 23.2 (2 H), 24.9 (3 H), 25.6 (3 H), 26.7 (2 H), 39.7 (2 H), 41.1 (2 H), 44.0, 111.9 (2 H), 113.6 (1 H), 120.6 (1 H), 124.4 (1 H), 124.4 (1 H), 124.5, 131.4, 135.1, 138.2, 141.7, 144.4, 146.8 (1 H).

Peltatol B (2): clear colorless oil; $[\alpha]_D$ −8.8° ($CHCl_3$, *c* 0.7); UV λ_{max} (MeOH) 281 (ε 4500) nm; IR (film) ν_{max} 3416 (broad), 2967, 2921, 1597, 1511, 1423, 1374, 1193, 1117, 1022 cm^{-1}; HREIMS *m/z* 626.4340 (calcd for $C_{42}H_{58}O_4$, 626.4335); ^1H NMR ($CDCl_3$) δ 1.23 (s, 3 H), 1.25 (s, 3 H), 1.47 (s, 3 H), 1.48 (s, 3 H), 1.56 (s, 6 H), 1.65 (s, 6 H), 1.52–1.83 (m, 8 H), 1.91 (m, 4 H), 2.01 (m, 4 H), 4.92 (dd, *J* = 17.5, 1.5 Hz, 1 H), 4.93 (dd, *J* = 17.5, 1.5 Hz, 1 H), 4.99 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.00 (dd, *J* = 11.0, 1.5 Hz, 1 H), 5.02–5.07 (m, 4 H), 5.33 (br, exch, 1 H), 5.41 (br, exch, 1 H), 5.46 (br, exch, 1 H), 5.86 (dd, *J* = 17.5, 11.0 Hz, 1 H), 5.89 (dd, *J* = 17.5, 10.5 Hz, 1 H), 6.39 (d, *J* = 2.0 Hz, 1 H), 6.71 (d, *J* = 2.0 Hz, 1 H), 6.82 (d, *J* = 2.0 Hz, 1 H), 6.95 (d, *J* = 8.5 Hz, 1 H), 6.98 (dd, *J* = 8.5, 2.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.9 (3 H), 15.9 (3 H), 17.7 (2 C; 3 H), 23.1 (2 C; 2 H), 24.8 (3 H), 24.9 (3 H), 25.7 (2 C; 3 H), 26.7 (2 C; 2 H), 39.7 (2 C; 2 H), 41.0 (2 H), 41.1 (2 H), 43.9, 44.0, 108.2 (1 H), 109.8 (1 H), 111.8 (2 H), 111.9 (2 H), 115.8 (1 H), 116.5 (1 H), 122.9 (1 H), 124.3 (2 C; 1 H), 124.3 (1 H), 124.4 (1 H), 131.3 (2 C), 132.2, 135.0 (2 C), 140.4, 140.5, 142.7, 143.0, 144.5, 144.7, 146.5 (1 H), 146.7 (1 H).

Peltatol C (3): clear colorless oil; $[\alpha]_D$ +6° ($CHCl_3$, *c* 0.5); UV λ_{max} (MeOH) 281 (ε 5500) nm; IR (film) ν_{max} 3415 (broad), 3082, 2966, 2922, 1598, 1511, 1432, 1192, 1016 cm^{-1}; HREIMS *m/z* 626.4335 (calcd for $C_{42}H_{58}O_4$, 626.4335); ^1H NMR ($CDCl_3$) δ 1.25 (s, 3 H), 1.34 (s, 3 H), 1.47 (s, 3 H), 1.50 (s, 3 H), 1.57 (s, 3 H), 1.58 (s, 3 H), 1.65 (s, 3 H), 1.66 (s, 3 H), 1.57–1.88 (m, 8 H), 1.93 (m, 4 H), 2.03 (m, 4 H), 4.96 (dd, *J* = 17.5, 1.0 Hz, 1 H), 5.01 (dd, *J* = 10.5, 1.0 Hz, 1 H), 5.02 (dd, *J* = 17.5, 1.0 Hz, 1 H), 5.08 (dd, *J* = 10.5, 1.0 Hz, 1 H), 5.02–5.08 (m, 4 H), 5.33 (br, exch, 2 H), 5.53 (br, exch, 1 H), 5.90 (dd, *J* = 17.5, 10.5 Hz, 1 H), 5.99 (dd, *J* = 17.5, 10.5 Hz, 1 H), 6.45 (d, *J* = 2.0 Hz, 1 H), 6.69 (d, *J* = 8.5 Hz, 1 H), 6.73 (d, *J* = 2.0 Hz, 1 H), 6.74 (dd, *J* = 8.5, 2.0 Hz, 1 H), 7.00 (d, *J* = 2.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.9 (2 C; 3 H), 17.6 (2 C; 3 H), 23.0 (2 H), 23.1 (2 H), 24.8 (2 C; 3 H), 25.6 (2 C; 3 H), 26.7 (2 C; 2 H), 39.6 (2 C; 2 H), 41.0 (2 H), 41.1 (2 H), 43.9, 44.00, 109.4 (1 H), 110.4 (1 H), 111.9 (2 H), 111.9 (2 H), 114.9 (1 H), 116.3 (1 H), 119.0 (1 H), 124.4 (3 C; 1 H), 124.5 (1 H), 131.3, 131.4, 132.7, 135.1 (2 C), 140.7, 141.7, 142.7, 144.4, 144.6, 146.0, 146.5 (1 H), 146.7 (1 H).

4-Nerolidylcatechol (4): light yellow oil; $[\alpha]_D$ +3.3° (CH_2Cl_2, *c* 3.9); UV λ_{max} (MeOH) 282 (ε 2000), 216 (ε 4700) nm; IR (film) ν_{max} 3377 (broad), 2967, 2922, 1603, 1519, 1434, 1374, 1282, 1112, 1000 cm^{-1}; HREIMS *m/z* 314.2272 (calcd for $C_{21}H_{30}O_2$, 314.2246); ^1H NMR ($CDCl_3$) δ 1.31 (s, 3 H), 1.51 (s, 3 H), 1.59 (s, 3 H), 1.64 (m, 1 H), 1.67 (s, 3 H), 1.71 (m, 1 H), 1.78 (m, 1 H), 1.84 (m, 1 H), 1.94 (m, 2 H), 2.03 (m, 2 H), 5.01 (dd, *J* = 17.6, 1.2 Hz, 1 H), 5.05 (dd, *J* = 10.7, 1.2 Hz, 1 H), 5.08 (m, 2 H), 5.97 (dd, *J* = 17.6, 10.7 Hz, 1 H), 6.74 (dd, *J* = 8.3, 2.0 Hz, 1 H), 6.78 (d, *J* = 8.3 Hz, 1 H), 6.83 (d, *J* = 2.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.8 (3 H), 17.6 (3 H), 23.1 (2 H), 24.9 (3 H), 25.6 (3 H), 26.7 (2 H), 39.6 (2 H), 41.1 (2 H), 43.8, 111.6 (2 H), 114.3 (1 H), 115.0 (1 H), 119.2 (1 H), 124.4 (1 H), 124.6 (1 H), 131.4, 135.0, 141.1, 141.3, 143.0, 147.1.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds 1, 2, and 3 (6 pages). Ordering information is given on any current masthead page.

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