Cytotoxic Activity of Tetraprenylphenols Related to Suillin, an Antitumor Principle from Suillus granulatus

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CYTOTOXIC ACTIVITY OF TETRAPRENYLPHENOLS RELATED TO SUILLIN, AN ANTITUMOR PRINCIPLE FROM *SUILLUS GRANULATUS*

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ABSTRACT.—Cytotoxic activity against three tumor cell lines (KB, P-388, and NSCLC-N6) has been determined for 14 natural and semisynthetic tetraprenylphenols. Structure-activity relationships have been examined.

The tetraprenylphenol suillin [**1**] (1) has been identified as the principle responsible for the cytotoxic activity of the lipid extract of the basidiomycete *Suillus granulatus* (L. ex Fr.) O. Kuntze (Boletaceae) (2). In addition to suillin, *S. granulatus* elaborates lesser amounts of the related compounds **2**, **4**, **9**, **11**, and **12** (3). The recent availability of substantial amounts of these tetraprenylphenols and the observation that suillin possesses significant in vivo antitumor activity against P-388 murine ascitic leukemia led us to extend the biological testing to the congeners of this fungal metabolite as well as to some derivatives. Of these, compounds **3**, **6**, and **10** were prepared as described in previous work, while **7**, **8**, and **14** were obtained by catalytic hydrogenation of **1**, **3**, and **13**, respectively. Finally, dimethoxysuillin [**5**] was isolated, along with **6**, from the products of the treatment of suillin with methyl sulfate in alkaline medium. The spectral properties of all the previously unreported semisynthetic compounds agreed with the assigned structures.

Cytotoxic activities of compounds **2**–**14** have been determined against the following cell lines: KB cells (human nasopharyngeal cancer), P-388 cells (murine leukemia), and NSCLC-N6 (human bronchopulmonary carcinoma) (4). The results (Table 1) may be summarized as follows:

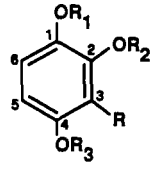
(a) Most of the compounds tested are significantly active ($IC_{50} \leq 5$ μg/ml) against at least one of the cell lines used.

(b) Methylation of the phenolic hydroxyls causes a marked decrease of the activity, as results from comparison of **5** and **6** with **1**.

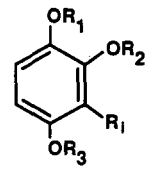
(c) Hydrogenation of the double bonds in the side chain also results in partial loss of activity (**7** and **8** should be compared with **1** and **3**, respectively). A higher degree of chain flexibility appears to be detrimental to the cytotoxicity, but this effect seems to be restricted to the portion of the chain proximal to the benzene nucleus. In fact, no substantial difference is observed between the activities of **13** and **14**.

(d) Location of the side chain also appears to play a role on the level of activity, which is higher when it is at position 6 rather than in position 3 (compare **10** and **3**).

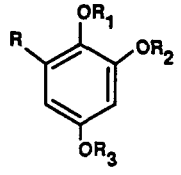
On the whole, these results confirm the biological importance of prenylphenols, a class of natural products which includes antimicrobial (3,5,6), antioxidant (7,8), allergenic (9,10), anti-inflammatory (11), and cyctotoxic (6) metabolites.



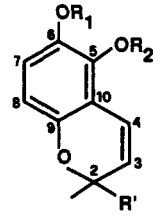
1 $R_1=R_2=H$, $R_3=Ac$
2 $R_1=R_3=H$, $R_2=Ac$
3 $R_1=R_2=R_3=Ac$
4 $R_1=R_2=H$, $R_3=Me$
5 $R_1=R_2=Me$, $R_3=Ac$
6 $R_1=R_2=R_3=Me$



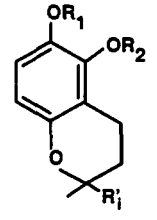
7 $R_1=R_2=H$, $R_3=Ac$
8 $R_1=R_2=R_3=Ac$



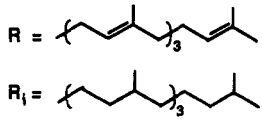
9 $R_1=Ac$, $R_2=R_3=H$
10 $R_1=R_2=R_3=Ac$
11 $R_1=Me$, $R_2=R_3=H$

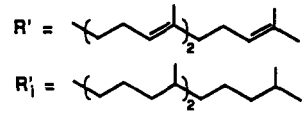


12 $R_1=R_2=H$
13 $R_1=R_2=Ac$



14 $R_1=R_2=Ac$





EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ms spectra were obtained at 70 eV on a Kratos MS-50 spectrometer. The nmr spectra were recorded at 250.13 (^{1}H) and 62.89 (^{13}C) MHz, using a Bruker AC-250 instrument equipped with a ^{13}C-^{1}H dual probe head and an Aspect 3000 data system. For both ^{1}H- and ^{13}C-nmr spectra, $CDCl_3$ was used as solvent and TMS as an internal standard. Chemical shifts are measured in δ (ppm). The 2D-nmr NOESY (12) experiment was run in C_6D_6 (a spectral width of 2100 Hz in both dimensions; 1K data points for 256 increments with 44 transients were used). Natural tetraprenylphenols **1**, **2**, **4**, **9**, **11**, and **12** were isolated from carpophores of *S. granulatus* according to the procedure described in previous work (2,3). Compounds **3**, **6**, **10**, and **13** were prepared from the appropriate natural products as described previously (2,3). The semisynthetic products **5**, **7**, **8**, and **14** were obtained as described below.

CYTOTOXICITY ASSAYS.—Cytotoxicity tests were performed in 96-well microplates (Falcon 3072, flat bottom microtest plate with lid). NSCLC cells (0.2×10^5), KB cells (0.1×10^5), or P388 cells (0.05×10^5) were placed in each well containing 50 μl of RPMI 1640 medium Gibco supplemented with 5% fetal calf serum, to which 100 IU penicillin/ml 100 μg streptomycin/ml, and 2 mM glutamine were added. Subsequently, 50 μl of the solution to be tested was added in decreasing concentrations using two wells for each dose. Microtest plates were then incubated for 72 h at 37° in 5% CO_2 in air. Cell proliferation was estimated by a colorimetric test (13); 10 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-

TABLE 1. Cytotoxicity of Tetraprenylphenols.[a]

Compound	Cell Line		
	KB IC_{50} (μg/ml)[b]	P-388 IC_{50} (μg/ml)[b]	NSCLC-N6 IC_{50} (μg/ml)[b]
1	0.7	0.8	1.0
2	2.5	0.9	2.9
3	2.0	5.0	1.9
4	n.a.(5.0)	2.8	>5.0
5	7.5	3.7	5.8
6	n.a.(10.0)	>10.0	>10.0
7	6.3	8.2	4.5
8	>10.0	>16.6	9.0
9	n.a.(5.0)	2.3	2.3
10	0.9	0.4	0.8
11	5.0	2.1	2.2
12	n.a.(5.0)	n.a.(5.0)	n.a.(5.0)
13	1.0	0.3	0.7
14	1.1	0.7	0.9

[a]Suillin [**1**] added for comparison.
[b]n.a.=not active at the concentration indicated (μg/ml).

diphenyltetrazolium bromide] (SIGMA) was added. After 4 h, the dark blue crystals, formed in mitochondria of living cells during the reduction of the MTT, were solubilized with 100 μl of isopropanoic acid. Microplates were read by ELISA using a multiskar Titertek multiscan MK II with a 570 nm filter. The optical density of the wells then enabled a dose/effect curve to be plotted and the IC_{50} (growth cell inhibition for each product relative to controls) to be determined.

PREPARATION OF **7**, **8**, AND **14**.—Compounds **1**, **9**, and **13** (30 mg each) in 95% EtOH were hydrogenated over 10% Pd/C to give the tetrahydroderivatives **7**, **8**, and **14**.

4-Acetoxy-1,2-dihydroxy-3-(3′,7′,11′,15′-tetramethylhexadecyl)benzene [**7**].—^{1}H nmr δ 0.84, 0.87 (each 6H, d, J=6 Hz) and 0.92 (3H, d, J=6 Hz) (H_3-16′ and Me-3′, -7′, -11′, or -15′), 1.10–1.50 (24H, m, H-3′, -7′, -11′, or -15′ and H_2-2′, -4′, -5′, -6′, -8′, -9′, -10′, -12′, -13′, or -14′), 2.31 (3H, s, Ac), 2.45 (2H, m, H_2-1′), 6.33 and 6.42 (2H, AB system, J=9 Hz, H-5, H-6); ^{13}C nmr ppm 171.4 (s, Ac), 143.4 (s, C-4), 142.5 (s, C-2), 141.0 (s, C-1), 122.3 (s, C-3), 112.8 and 112.7 (d, C-5 or C-6) 39.3, 37.4, 37.2, 37.1, 36.1, and 36.0 (7C, t, C-2′, -4′, -6′, -8′, 10′, -12′, or -14′), 33.0 (d, C-3′), 32.8 (2C, d, C-7′ or C-11′), 27.9 (d, C-15′), 24.8 and 24.5 (3C, t, C-5′, -9′ or -13′), 22.7, 22.6 (q, C-16′ or Me-15′), 22.1 (t, C-1′), 20.9 (q, Ac), 19.7, 19.5, and 19.4 (q, Me-3′, -7′ or -11′); hrms [M]$^+$ 448.3546 ($C_{28}H_{48}O_4$ requires 448.3552); ms *m/z* 448 (18%), 406 (100%), 364 (1%), 181 (7%), 152 (8%), 139 (11%), 126 (5%), 71 (7%), 69 (7%), 57 (7%).

1,2,4-Triacetoxy-3-(3′,7′,11′,15′-tetramethylhexadecyl)benzene [**8**].—^{1}H nmr δ 0.84, 0.87 (each 6H, d, J=6 Hz) and 0.92 (3H, d, J=6 Hz) (H_3-16′ and Me-3′, -7′, -11′, or -15′), 1.10–1.40 (24H, m, H-3′, -7′, -11′, or -15′ and H_2-2′, -4′, -5′, -6′, -8′, -9′, -10′, -12′, -13′, or -14′), 2.25, 2.30 and 2.31 (9H, 3×Ac), 2.40 (2H, m, H_2-1′), 6.97 and 7.07 (2H, AB system, J=9 Hz, H-5, H-6); ^{13}C nmr ppm 168.9, 168.0, and 167.8 (s, 3×Ac), 146.6 (s, C-4), 141.1 (s, C-2), 140.2 (s, C-1), 129.7 (s, C-3), 120.5 and 120.2 (d, C-5 or C-6), 39.3, 37.3, 36.9, 36.1, and 36.0 (7C, t, C-2′, -4′, -6′, -8′, -10′, -12′, or -14′), 33.0 (d, C-3′), 32.7 (2C, d, C-7′ or C-11′), 27.9, 24.7, and 24.4 (t, C-5′, -9′, or-13′), 22.8 (t, C-1′), 22.7, 22.6 (q, C-16′ or Me-15′), 20.8, 20.6, and 20.2 (q, 3×Ac), 19.6, 19.5, and 19.4 (q, Me-3′, -7′, or 11′); hrms [M]$^+$ 532.3760 ($C_{32}H_{52}O_6$ requires 532.3764); ms *m/z* 532 (2%), 490 (10%), 448 (35%), 406 (100%), 390 (2%), 320 (3%), 306 (0.8%), 292 (0.8%), 278 (0.8%) 264 (0.8%), 250 (0.8%), 181 (9%), 139 (49%), 71 (20%), 69 (9%), 57 (35%).

5,6-Diacetoxy-2-methyl-2-(4′,8′,12′-trimethyltridecyl)cromane [**14**].—^{1}H nmr δ 0.83, 0.84 (each 3H, d, J=6 Hz), 0.86 (6H, d, J=6 Hz) (Me-4′, -8′, or -12′), 1.20–1.52 (21H, m, H-4′, -8′, or -12′ and H_2-1′, -2′, -3′, -5′, -6′, -7′, -9′, -10′, or -11′), 1.26 (3H, s, Me-2), 1.75 (2H, m, H_2-3), 2.25, 2.30 (6H, s, 2×Ac), 2.55 (2H, t, H_2-4), 6.69 and 6.90 (2H, AB system, J=9 Hz, H-7, H-8); ^{13}C nmr ppm 169.0, 167.9 (s, 2×Ac), 152.4 (s, C-9), 140.3 (s, C-5), 135.8 (s, C-6), 121.0 (d, C-7), 115.7 (s, C-10), 114.9 (d, C-8), 76.5 (s, C-2), 39.9 (t, C-1′), 39.3 (t, C-11′), 37.4 (4C, t, C-3′, -5′, -7′, or -9′), 32.7 (2C, d, C-4′, C-8′), 29.6 (t,

C-3), 27.9 (d, C-12′), 24.8 (t, C-10′), 24.4 (t, C-6′), 23.9 (q, Me-2), 22.7 (2C, q, Me-12′, C-13′), 21.0 (t, C-2′), 20.6 and 20.3 (q, 2×Ac), 19.6 (2C, q, Me-4′, -8′), 17.4 (t, C-4); hrms $[M]^+$ 488.3512 ($C_{30}H_{48}O_5$ requires 488.3501); ms *m/z* 488 (4%), 446 (41%), 404 (100%), 380 (4%), 223 (11%), 179 (18%), 169 (26%), 151 (22%), 139 (63%), 111 (15%), 82 (33%), 71 (11%), 69 (15%).

PREPARATION OF **5**.—Suillin (50 mg) was methylated according to the procedure described in previous work (3). Si gel fractionation of the crude product yielded, in addition to **6**, lesser amounts of **5**.

4-Acetoxy-3-geranylgeranyl-1.2-dimethoxybenzene [**5**].—^{1}H nmr ($CDCl_3$) δ 1.58 (6H, s), 1.59 (3H, s) (Me-7′, -11′, or -15′), 1.67 (3H, s, H_3-16′), 1.75 (3H, s, Me-3′), 1.88–2.10 (12H, m, H_2-4′, -5′, -8′, -9′, -12′, or -13′), 2.27 (3H, s, Ac), 3.28 (2H, d, J=7 Hz, H_2-1′), 3.60 and 3.64 (each 3H, s, 1-OMe or 2-OMe), 5.09 (4H, bs, H-2′, -6′, -10′, or -14′), 6.76 (2H, bs, H-5 and H-6); ^{1}H nmr (C_6H_6) δ 1.55, 1.56, 1.59, 1.67, and 1.68 (each 3H, s, Me-3′, -7′, -11′, -15′, or H_3-16′), 1.87 (3H, s, Ac), 2.00–2.25 (12H, m, H_2-4′, -5′, -8′, -9′, -12′, or 13′), 3.27 (3H, s, 1-OMe), 3.52 (2H, d, J=7 Hz, H_2-1′), 3.72 (3H, s, 2-OMe), 5.23 (3H, m, H-6′, -10′, or -14′), 5.44 (1H, t, H-2′), 6.38 and 6.79 (2H, AB system, J=9 Hz, H-6 and H-5, respectively); observed cross-correlation in the NOESY spectrum (250 MHz, C_6D_6) δ 3.27/3.72, 3.27/6.38, 6.38/6.79; ^{13}C nmr ($CDCl_3$) ppm 169.9 (s, Ac), 150.8 (s, C-2), 147.6 (s, C-4), 142.7 (s, C-1), 135.4, 135.0 134.9, and 131.2 (s, C-3′, -7′, -11′, or 15′), 128.4 (s, C-3), 124.4, 124.2, and 124.1 (d, C-6′, -10′, or -14′), 122.0 (d, C-2′), 117.4 (d, C-5), 109.7 (d, C-6), 60.8 and 55.9 (2C, q, 1-OMe or 2-OMe), 39.7 (3C, t, C-4′, -8′, or -12′), 26.7 and 26.6 (3C, t, C-5′, -9′, or -13′), 25.7 (q, C-16′), 23.8 (t, C-1′), 20.9 (q, Ac), 17.7 (q, Me-15′), 16.2 and 16.0 (3C, q, Me-3′, -7′, or -11′), hrms $[M]^+$ 468.3231 ($C_{30}H_{44}O_4$ requires 468.3239); ms *m/z* 468 (8%), 453 (0.8%), 426 (11%), 398 (19%), 264 (39%), 259 (16%), 221 (72%), 209 (43%), 205 (43%), 167 (100%), 135 (16%), 121 (17%), 109 (16%), 95 (16%), 81 (34%), 69 (87%).

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