Gibbilimbols A−D, Cytotoxic and Antibacterial Alkenylphenols from *Piper gibbilimbum*

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Received November 26, 1997

Fractionation of the petroleum ether extract from the leaves of *Piper gibbilimbum* collected in Papua New Guinea afforded four new alkenylphenols, gibbilimbols A−D (**1**−**4**). The structures of the isolates were elucidated by spectroscopic methods, mainly 1D- and 2D-NMR spectroscopy. Gibbilimbols A−D were found to be toxic to brine shrimp with an LC_{50} of approximately 5 μg/mL. Gibbilimbols A−D were further found to be cytotoxic toward KB nasopharyngal carcinoma cells (ED_{50} 7.8−2.1 μg/mL). All isolates also showed antibacterial activity toward *Staphylococcus epidermidis* and *Bacillus cereus*.

In our continuing study on plants used in the traditional medicine of Papua New Guinea (PNG), we have currently investigated the leaves of *Piper gibbilimbum* C.DC. (Piperaceae). The leaves of *P. gibbilimbum*, a scrambling shrub or small tree, are used in PNG as an antiseptic to heal abscess and ulceration of the skin and to treat fever.[1,2] The juice from the heated bark is also used to treat cancer and internal sores.[3] We here wish to report the isolation and structure elucidation of four new alkenylphenols, gibbilimbols A−D (**1**−**4**), together with an assessment of their antibacterial and cytotoxic potential.

Air-dried and powdered leaves (1.2 kg) of *P. gibbilimbum* were extracted successively with petroleum ether, CH_2Cl_2, MeOH, and 70% MeOH/H_2O. The crude petroleum ether extract showed activity in the brine shrimp lethality assay (LD_{50} < 1000 μg/mL). Chromatographic separations of the petroleum ether extract by a combination of methods (VLC and HPLC) over silica gel and ODS silica gel led to the isolation of gibbilimbols A−D (**1**−**4**).

Compound **1** was obtained as a clear oil. The HREIMS of **1** gave a $[M]^+$ peak at *m*/*z* 232.1816, consistent with the molecular formula $C_{16}H_{24}O$. Its IR spectrum contained absorptions due to hydroxyl (3373 cm^{-1}), and the UV spectrum exhibited a maximum at 279 nm. Of the five degrees of unsaturation implied by the molecular formula, all could be accounted for by examination of the ^{13}C NMR spectral data: as a para-substituted phenol moiety (δ 153.4, 115.0, 129.5, 134.9) and two olefinic carbons forming one double bond (Table 2).

The ^1H NMR data of compound **1** (Table 1) also showed the presence of an OH group (δ 4.60, 1H, br s, exchangeable with D_2O) and four A_2B_2-type aromatic protons (δ 6.75 and 7.05, each 2H, d, J = 8.5 Hz), confirming the presence of a para-substituted phenol. The remaining ^1H NMR signals showed the presence of a double bond (δ 5.41, 2H, H-4′ and H-5′) also evident in the ^{13}C NMR (δ 129.8, d and 131.0, d), a benzylic methylene group (δ 2.54, 2H, t, J = 7.7 Hz), two allylic methylenes (δ 2.00, 4H), four additional methylenes (δ 1.64, 2H, m and 1.29, 6H), and one methyl (δ 0.89, 3H, t, J = 6.7 Hz). Together, these data indicated a decenyl chain, which was confirmed by DQF−COSY, HMQC, and HMBC experiments.

Table 1. ^1H NMR Spectral Data of Gibbilimbols A−D (**1**−**4**) (300 MHz, $CDCl_3$, δ ppm, J Hz)

	compound[a]			
proton(s)	**1**	**2**	**3**	**4**
H-2,6	6.75, d, 8.5	6.75, d, 8.4	6.75, d, 8.5	6.75, d, 8.4
H-3,5	7.05, d, 8.5	7.05, d, 8.4	7.05, d, 8.5	7.05, d, 8.4
H-1′	2.54, t, 7.7	2.60, t, 7.8	2.55, t, 7.7	2.61, t, 7.8
H-2′	1.64, m	2.27, m	1.67, obs	2.27, m
H-3′	2.00, obs	5.43, obs	2.00, obs	5.43, obs
H-4′	5.41, obs	5.43, obs	5.42, obs	5.43, obs
H-5′	5.41, obs	1.98, m	5.42, obs	1.99, m
H-6′	2.00, obs	1.27, obs	2.00, obs	1.32, obs
H-7′	1.29, obs	1.27, obs	1.38, m	1.32, obs
H-8′	1.29, obs	1.27, obs	0.90, t, 7.3	0.90, t, 7.0
H-9′	1.29, obs	1.27, obs		
H-10′	0.89, t, 6.7	0.89, t, 6.7		
OH	4.60 br s	nd	4.58 br s	4.72 br s

[a] nd: not detected. obs: obscured.

Table 2. ^{13}C NMR Spectral Data of Gibbilimbols A−D (**1**−**4**) (75.5 MHz, $CDCl_3$, δ (ppm))

	compound			
carbon	**1**	**2**	**3**	**4**
C-1	153.4 s	153.4 s	153.4 s	153.4 s
C-2,6	115.0 d	115.0 d	115.0 d	115.0 d
C-3,5	129.5 d	129.5 d	129.5 d	129.5 d
C-4	134.9 s	134.5 s	134.9 s	134.5 s
C-1′	34.4 t	35.2 t	34.4 t	35.2 t
C-2′	31.6 t	34.7 t	31.6 t	34.7 t
C-3′	32.0 t	129.3 d	32.0 t	129.3 d
C-4′	129.8 d	131.2 d	130.0 d	131.1 d
C-5′	131.0 d	32.6 t	130.7 d	32.2 t
C-6′	32.6 t	29.5[a] t	34.7 t	31.7 t
C-7′	29.3 t	28.8[a] t	22.7 t	22.1 t
C-8′	31.4 t	31.8 t	13.6 q	13.9 q
C-9′	22.5 t	22.6 t		
C-10′	14.1 q	14.1 q		

[a] Interchangeable.

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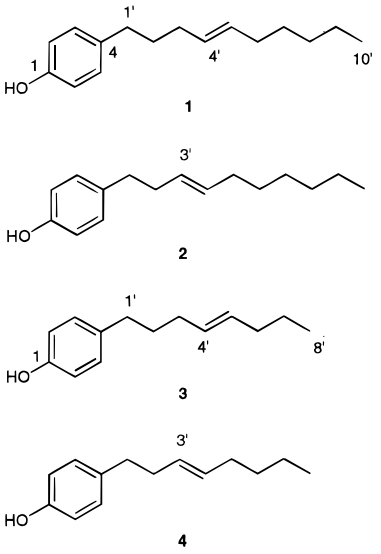
S0163-3864(97)00529-6 CCC: $15.00
Published on Web 07/03/1998



The position of the double bond was also evident from the DQF-COSY experiment: correlation cross-peaks between the benzylic protons at δ 2.54 (H_2-1′) with the methylene protons at δ 1.64 (H_2-2′), which in turn showed cross-peaks to the allylic protons at δ 2.00 (H_2-3′ and H_2-6′), indicated the double bond to be at C-4′. The allylic protons at δ 2.00 further showed a correlation cross-peak to the overlapped double-bond protons at δ 5.41 (H-4′ and H-5′). Unambiguous confirmation of the structure was obtained from the results of a HMBC experiment.

The stereochemistry of the double bond was elucidated by comparing the ^{13}C NMR chemical shifts of the allylic carbons (δ 32.0 and δ 32.6) with the chemical shifts calculated for 4′(*E*)-decenylbenzene (δ 32.1 and δ 33.3) and 4′(*Z*)-decenylbenzene (δ 27.6 and δ 27.8): the values found in **1** indicated the double bond to be trans.[4] This assignment was confirmed by the IR spectrum, which showed a strong absorption at 967 cm^{-1}, as expected for a trans double bond.[5] Compound **1** is therefore 4-(4′(*E*)-decenyl)phenol, for which we propose the name gibbilimbol A.

The spectral data of compound **2** indicated it to be closely related to compound **1**. The EIMS gave the same $[M]^+$ peak at *m*/*z* 232 consistent with the same molecular formula, $C_{16}H_{24}O$. The ^{1}H and ^{13}C NMR data were very similar and showed again the presence of a para-substituted phenol with a decenyl side chain (Tables 1 and 2). The only major differences were observed in the ^{1}H spectrum for the allylic protons; δ 1.98, 2H and 2.27, 2H in compound **2** compared to δ 2.00, 4H in compound **1**. This suggested a different position for the double bond. This was confirmed by a DQF-COSY experiment, which showed a correlation from the benzylic protons at δ 2.60 (H_2-1′) to the allylic protons at δ 2.27 (H_2-2′), which in turn showed a cross-peak to the olefinic group at δ 5.43 (H-3′ and H-4′), indicating the double bond to be at C-3′. These deductions were confirmed by HMQC and HMBC experiments. The stereochemistry of the double bond was assigned to be trans by interpretation of the ^{13}C NMR chemical shifts and IR spectral data as outlined above. The ^{13}C NMR chemical shifts of the allylic carbons (34.7 and 32.6) were compared with the chemical shifts calculated for 3′(*E*)-decenylbenzene (34.8 and 33.2) and 3′(*Z*)-decenylbenzene (29.7 and 27.8).[4] On the basis of these data, the structure of compound **2** was assigned as 4-(3′(*E*)-decenyl)phenol, for which we propose the name gibbilimbol B.

Table 3. Biological Activities of Gibbilimbols A−D (**1**−**4**)

compd	*Artemia salina* toxicity (LD_{50} μg/mL)	KB cell cytotoxicity (ED_{50} μg/mL)	antimicrobial activity (MIC in μg/mL)	
			Staphylococcus epidermidis	*Bacillus cereus*
1	5.3	4.4	4.0	4.0
2	4.9	3.9	2.0	4.0
3	5.5	7.8	2.0	2.0
4	5.0	2.1	2.0	2.0
chloramphenicol			8.0	4.0

The spectral data of compounds **3** and **4** appeared virtually identical to that of compounds **1** and **2**, respectively, the major difference being in their EIMS where a $[M]^+$ peak at *m*/*z* 204 for both compounds was observed, consistent with the molecular formula $C_{14}H_{20}O$. The spectral data for compounds **3** and **4** confirmed as well the presence of a para-substituted phenol (Tables 1 and 2). This implied the presence of an eight- instead of a 10-carbon side chain, which was confirmed by DQF-COSY, HMQC, and HMBC experiments. The stereochemistry of the double bond was assigned to be trans by interpretation of the ^{13}C NMR chemical shifts and IR spectral data as outlined above. Compound **3** was assigned as 4-(4′(*E*)-octenyl)phenol, the octenyl analogue of compound **1**, for which we propose the name gibbilimbol C. Compound **4** was assigned as 4-(3′(*E*)-octenyl)-phenol, the octenyl analogue of compound **2**, for which we propose the name gibbilimbol D.

Despite the fact that the genus *Piper* has been intensly studied, alkenylphenols such as gibbilimbols A−D have rarely been isolated.[6] In fact, there has been only one report of the occurrence of a C-16 alkenylphenol in *P. hispidum*.[7] The brine shrimp lethality (*Artemia salina*) and the cytotoxic activity toward KB nasopharyngal carcinoma cells of gibbilimbols A−D were evaluated (Table 3). All gibbilimbols were found to be active in a close range of 2−8 μg/mL in both assays. Further, a qualitative assessment of antibacterial potential against *Staphylococcus epidermidis*, *Bacillus cereus*, and *Pseudomonas aeruginosa* was evaluated using the paper disk diffusion technique. Small zones of growth inhibition (<2 mm at 60 μg/disk) were observed for all isolates with *S. epidermidis* and *B. cereus*, while no inhibition of growth was observed for *P. aeruginosa*. The MIC values for *S. epidermidis* and *B. cereus* were determined by the broth dilution method (Table 3). All isolates were found to possess MIC values between 2 and 4 μg/mL. The leaves of *P. gibbilimbum* are used in PNG as an antiseptic to heal abscess and ulceration of the skin. The antibacterial activity of gibbilimbols A−D reported here supports this traditional use of *P. gibbilimbum*.

Experimental Section

General Experimental Procedures. IR spectra were measured on a Perkin-Elmer 2000 FT-IR spectrometer as liquid films on NaCl tablets. UV spectra were recorded in MeOH using an Uvikon 930 spectrophotometer. EIMS spectra were taken on a Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV. NMR spectra were measured employing a Bruker AMX-300 instrument operating at a basic frequency of 300

MHz, using the solvent as internal standard ($CDCl_3$, at δ 7.26 and δ 77.0). Silica (Si gel 60, 5−40 μm, Merck) and RP-18 (40−63 μm, Baker) were used for VLC (column 20 × 6.5 cm, vacuum by H_2O aspiration). HPLC separations were performed with a Waters model 590 pump connected to a Knauer differential refractrometer (column Spherisorb ODS II, 5 μm, 250 × 8 mm, Knauer). All solvents were HPLC grade.

Plant Material. Leaves of *P. gibbilimbum* were collected near Goroka, Eastern Highlands Province of PNG, during September 1988.[8] Herbarium specimens are deposited at the Herbarium (ZT 11812) ETH, Zürich, Switzerland, as well as at UPNG Herbarium, Port Moresby, PNG, and at National Herbarium in Lae, PNG.

Brine Shrimp Lethality Bioassay. The brine shrimp (*Artemia salina*) lethality was performed as previously described.[9]

Cytotoxicity Testing. Cytotoxic potential was assessed using cultured KB (human nasopharyngal carcinoma) cells as previously described.[10,11]

Antibacterial Assays. Paper disk diffusion assays were used as a qualitative assessment of antibacterial potential of the isolates and extracts.[12] *S. epidermidis*, *B. cereus*, and *P. aeruginosa* were used to test antibacterial activity. Minimum inhibitory concentration (MIC) values were determined of active isolates by the broth dilution method using an inoculum of 10^4−10^5 cells/mL. The results were read after 24 h incubation at 37 °C. All bacterial strains were grown in BBL nutrient broth (Becton & Dickinson Co. 11479).

Extraction and Isolation. Air-dried and powdered leaves (1.2 kg) were successively percolated with petroleum ether, CH_2Cl_2, MeOH, and 70% MeOH/H_2O at room temperature. A portion of the petroleum ether extract (10 g) was applied to VLC. Elution over Si gel with hexane containing increasing amounts of EtOAc and final washing with MeOH yielded 30 fractions each of 100−180 mL. The fractions were combined, based on TLC similarities, to yield nine combined fractions. Fraction 5 (1.8 g) was separated by VLC over ODS, using MeOH−H_2O (9:1−10:0) mixtures as eluents. TLC and ^{1}H NMR investigations of these fractions indicated fraction 1 (79 mg) to be of further interest. Reversed-phase HPLC separation of this fraction with MeCN−H_2O 3:1 as an eluent gave two pure components, compound **1** (2.8 mg) and compound **2** (5.3 mg), as well as a fraction containing two additional components. This fraction was further purified by reversed-phase HPLC, using a mixture of MeOH−H_2O (78:22) as eluent, to give compound **3** (10.6 mg) and compound **4** (11.5 mg).

Gibbilimbol A (1): clear oil (2.8 mg, 0.028% of the crude extract); UV λ_{max} (MeOH) 279 nm (log ϵ = 3.36), 224 nm (log ϵ = 4.01); IR ν_{max} (film) 3373, 3020, 2957, 2926, 2855, 1614, 1598, 1549, 1378, 1017, 968 cm^{-1}; EIMS *m/z* (rel int) [M$^+$] 232 (19), 133 (30), 121 (16), 120 (100), 107 (68); HREIMS measurement for $C_{16}H_{24}O$ 232.1816 (Δ = 0.1 mmu); ^{1}H and ^{13}C NMR see Tables 1 and 2.

Gibbilimbol B (2): yellowish oil (5.3 mg, 0.053% of the crude extract); UV λ_{max} (MeOH) 279 nm (log ϵ = 3.21), 224 nm (log ϵ = 3.96); IR ν_{max} (film) 3355, 3022, 2957, 2854, 1614, 1599, 1514, 1455, 1235, 967 cm^{-1}; EIMS *m/z* (rel int) [M$^+$] 232 (12), 204 (8), 133 (5), 120 (15), 107 (100), 77 (10); HREIMS measurement for $C_{16}H_{24}O$ 232.1798 (Δ = 0.1 mmu); ^{1}H and ^{13}C NMR see Tables 1 and 2.

Gibbilimbol C (3): clear oil (10.6 mg, 0.106% of the crude extract); UV λ_{max} (MeOH) 279 nm (log ϵ = 3.30), 224 nm (log ϵ = 3.91); IR ν_{max} (film) 3373, 3015, 2957, 2926, 2855, 1614, 1598, 1515, 1456, 1239, 967 cm^{-1}; EIMS *m/z* (rel int) [M$^+$] 204 (15), 133 (28), 120 (99), 107 (100), 77 (18), 55 (15); HREIMS measurement for $C_{14}H_{20}O$ 204.1526 (Δ = 0.1 mmu); ^{1}H and ^{13}C NMR see Tables 1 and 2.

Gibbilimbol D (4): clear oil (11.5 mg, 0.115% of the crude extract); UV λ_{max} (MeOH) 279 nm (log ϵ = 3.21), 224 nm (log ϵ = 3.86); IR ν_{max} (film) 3333, 3023, 2957, 2926, 2855, 1614, 1599, 1514, 1454, 1235, 968 cm^{-1}; EIMS *m/z* (rel int) [M$^+$] 204 (19), 133 (3), 120 (7), 107 (100), 77 (12); HREIMS measurement for $C_{14}H_{20}O$ 204.1524 (Δ = 0.1 mmu); ^{1}H and ^{13}C NMR see Tables 1 and 2.

Acknowledgment. This research was supported in part by the Swiss National Science Foundation. One of us (P.M.) was supported by an Erasmus Fellowship (ICP 95 I-1241/12). We are in debt to Dr. C. A. J. Erdelmeier for collection of the plant materials, to Dr. M. Baltisberger (ETH Zurich) for preparation of herbarium specimens and for collection of plant materials, and to Mr. P. Katik (National Herbarium, Lae) for his valuable help concerning botanical matters. We thank Dr. O. Zerbe for assistance with NMR measurements. Our thanks are also due to Mr. M. Wasescha and Mr. W. Schühly (ETH Zurich) for valuable help with the antibacterial and cytotoxic testing. We also thank Mr. R. Häfliger (ETH Zurich) for recording mass spectra and Dr. E. Zass (ETH Zurich) for performing literature searches.

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NP970529I