Antimicrobial Activity of 8-Alkyl- and 8-Phenyl-Substituted Berberines and Their 12-Bromo Derivatives

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The 8-alkyl- (**3**−**6**), 8-phenyl- (**7**), 12-bromo- (**8**), 8-alkyl-12-bromo- (**9**−**12**), and 12-bromo-8-phenyl- (**13**) berberine derivatives were prepared and tested for their antimicrobial activity in vitro to evaluate structure−activity relationships. Introduction of the alkyl or phenyl group and the bromine atom into the C-8 and C-12 positions of berberine (**1**), respectively, led to significant increases of the antimicrobial activity. In both the 8-alkyl- and 8-alkyl-12-bromo-berberines (**3**−**6** and **9**−**12**, respectively), the antibacterial activity increased as the length of the aliphatic chain increased. The exception was the activity against *Candida albicans* and *Escherichia coli*, which did not always increase as the alkyl side chain lengthened. Among the compounds tested, 12-bromo-8-*n*-hexylberberine (**12**) was 64, 256, 128, 16, and 32 times more active against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enteritidis*, *E. coli*, and *C. albicans*, respectively, in comparison to the clinically used berberine. Compound **12** was also found to be 8, 16, and 128 times more active against *S. aureus*, *S. enteritidis*, and *C. albicans*, respectively, than kanamycin sulfate, but was of the same order of activity against *B. subtilis*, and only one-fourth as active against *E. coli*.

In Japan and other Asian countries, berberine (**1**) and the extracts of coptis rhizome (the rhizome of *Coptis japonica* Makino or other species of the same genus) and phellodendron bark (the bark of *Phellodendron amurense* Ruprecht or other species of the same genus) are used for treating diarrhea and other gastrointestinal diseases.[1] We reported previously on the antimicrobial activities of the 13-alkyl substituted analogues of **1** against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.[2,3]

In a recent paper, the antibacterial activity of these13-alkyl derivatives has also been studied against *Bacillus subtilis* and *Salmonella enteritidis*.[4] Additional berberine derivatives have been prepared and in the present paper, the effects of 8-alkyl and 8-phenyl groups and/or a 12-bromo substituent on antimicrobial activity are described.

The 8-alkyl- (**3**−**6**), 8-phenyl- (**7**), 12-bromo- (**8**), 8-alkyl-12-bromo- (**9**−**12**), and 12-bromo-8-phenyl- (**13**) berberines were synthesized as presented in Scheme 1 for investigation of activity against Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*S. enteritidis* and *E. coli*) bacteria as well as the fungus *C. albicans*. Structures **3**−**13** were confirmed by ^{1}H NMR (Table 1), including NOESY spectra and HPLC, LSIMS, and HRLSIMS data (Table 2).

The antimicrobial activity of compounds **3**−**6** was compared with previous results[3,4] for berberine (**1**) and its simple 13-alkyl analogues. As a result, the antimicrobial activity of individual 8-alkyl-substituted berberines was generally higher than that of the corresponding 13-alkyl-berberines[3,4] (compare 8-alkyl-berberines **3**, **4**, **5**, and **6** in Table 3 with the corresponding 13-alkyl derivatives in our previous papers[3,4]). The activities of the 8-alkyl derivatives **3**−**6** against *S. aureus*, *B. subtilis*, and *S. enteritidis* increased as the length of the alkyl chain at the C-8 position increased, paralleling the retention times of the 8-alkyl-berberines (**3**−**6**) in reversed-phase HPLC (Table 2). This profile, however, is absent against *E. coli* and *C. albicans*. These results are similar to those previously obtained for the 13-alkyl-berberines.[3,4] The correlation of alkyl chain length with increased activity would suggest that lipophilicity contributes to activity.

The antimicrobial activities of the 12-bromo derivatives (**8**−**11** and **13**) were more potent than those of the corresponding nonbrominated precursors (compare **8**, **9**, **10**, **11**, and **13** with **1**, **4**, **5**, **6**, and **7**, Table 3). Again, the activities and the retention times of the 8-alkyl-12-bromo-berberines **9**−**12** increased with increasing length of the alkyl side-chain (**9**−**12**, Tables 2 and 3).

Among the compounds tested, 12-bromo-8-*n*-hexylberberine (**12**) exhibited the highest activity against all strains tested (Table 3). This compound showed stronger activity against *S. aureus*, *B. subtilis*, *S. enteritidis*, *E. coli*, and *C. albicans* than clinically used berberine (**1**) by factors of 64, 256, 128, 16, and 32, respectively.

Derivative **12** also displayed higher activity against *S. aureus*, *S. enteritidis*, and *C. albicans* than kanamycin sulfate (KA) by factors of 8, 16, and 128, respectively.

This compound also demonstrated the same order of activity against *B. subtilis* as did KA, but only one-fourth the activity against *E. coli*. 12-Bromo-8-*n*-butylberberine (**11**) exhibited significant activity against all the tested microorganisms but *E. coli*.

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Scheme 1. Preparations of 8-alkyl- (**3**−**6**), 8-phenyl- (**7**), 12-bromo- (**8**), 8-alkyl-12-bromo- (**9**−**12**), and 12-bromo-8-phenyl- (**13**) berberine chlorides.

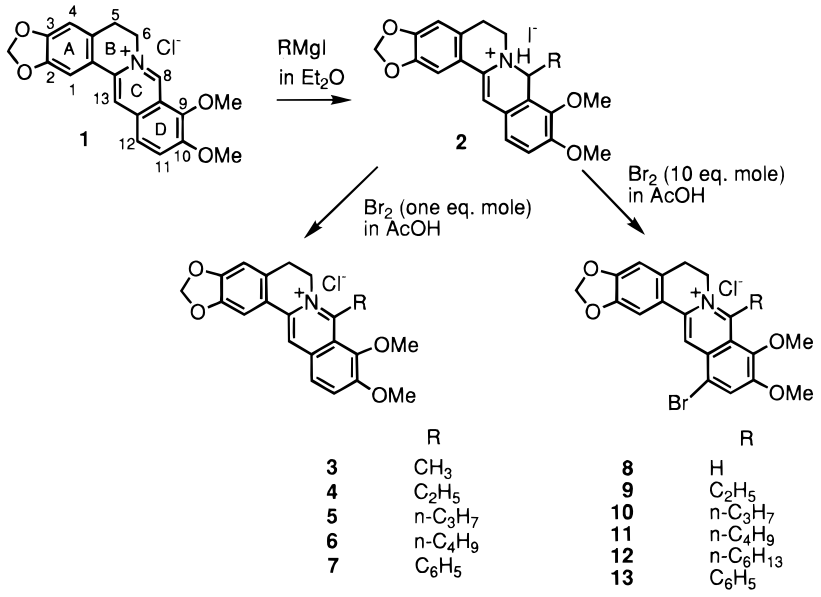

Table 1. ^{1}H NMR Data of 8-Alkyl- (**3**−**6**), 8-Phenyl- (**7**), 12-Bromo- (**8**), 8-Alkyl-12-bromo- (**9**−**12**), and 12-Bromo-8-phenyl- (**13**) berberines[a]

compd	1-H	4-H	5-H	6-H	8-H or 8-alkyl or 8-phenyl	OCH_2O	9-OMe	10-OMe	11-H	12-H	13-H
3	7.73	7.10	3.13[b]	4.75[b]	3.41	6.10	4.06	3.95	8.18[d]	8.02[d]	8.79
4	7.60	6.95	3.20[b]	4.80[b]	1.58 (3H)[c] 3.90 (2H, br)	6.17	4.16	4.11	8.08[d]	7.99[d]	8.55
5	7.60	6.95	3.20[b]	4.80[b]	1.27 (3H)[c] 3.83 (2H, br)	6.10	4.15	4.11	8.08[d]	7.99[d]	8.55
6	7.59	6.95	3.20[b]	4.80[b]	1.11 (3H)[c] 1.70 (2H, m) 1.89 (2H, m) 3.85 (2H, br)	6.10	4.14	4.11	8.08[d]	7.99[d]	8.55
7	7.68	6.89	3.04[b]	4.35[b]	7.56 (Ar-2H, m) 7.69 (Ar-3H, m)	6.11	3.28	4.04	8.12[d]	8.10[d]	8.80
8	7.68	6.98	3.27[b]	4.95[b]	9.86	6.13	4.22	4.12	8.43		8.61
9	7.77	7.14	3.17[b]	4.85[b]	1.47 (3H)[c] 3.78 (2H, br)	6.18	4.09	4.11	8.44		8.55
10	7.77	7.14	3.15[b]	4.85[b]	1.17 (3H)[c] 1.84 (2H, m) 3.72 (2H, br)	6.18	4.07	4.11	8.44		8.55
11[e]	7.76	7.14	3.18[b]	4.84[b]	1.02 (3H)[c] 1.61 (2H, m) 1.79 (2H, m) 3.75 (2H, br)	6.18	4.05	4.11	8.44		8.55
12[e]	7.77	7.14	3.16[b]	4.84[b]	0.92 (3H)[c] 1.34 (4H, m) 1.59 (2H, m) 1.80 (2H, m) 3.75 (2H, br)	6.18	4.05	4.11	8.44		8.55
13	7.92	7.06	3.01[b]	4.24[b]	7.64 (Ar-2H, m) 7.67 (Ar-3H, m)	3.16	4.03	8.59	8.67		6.20

[a] CD_3OD; δ ppm, 500 MHz. [b] Triplet, $J = 6.0$ Hz. [c] Triplet, $J = 7.0$ Hz. [d] Doublet, $J = 9.0$ Hz. [e] In DMSO-d_6.

In conclusion, introduction of hydrocarbon groups at C-8 of berberine increased the antimicrobial activity. A bromo substituent at C-12 even argumented this effect. 12-Bromo-substituted analogues of the 8-alkyl- and 8-phenyl-berberines showed greater activity against the microorganisms tested than did the nonbrominated derivatives.

Substitution of the hydrogen at the C-8 position of berberine with alkyl groups led to higher activity than did substitution at C-13. 12-Bromo-8-*n*-hexylberberine (**12**) exhibited the highest activity against all strains tested, except for *E. coli*, being a far stronger inhibitor than berberine (**1**) and KA. 12-Bromo-8-*n*-butylberberine (**11**) exhibited significant activity against the microorganisms tested except for *E. coli*. We conclude that 12-bromo-8-*n*-butyl- and 12-bromo-8-*n*-hexyl-berberines (**11** and **12**) may deserve greate clinical attention than berberine (**1**).

Experimental Section

General Methods. Melting points were determined on an IA 9100 (Aldrich) electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian VXR-500 (500 MHz) using TMS as a internal standard and CD_3OD or DMSO-d_6 as solvent. MS were determined on a Hitachi M-4100

Table 2. Physical, HPLC Analysis, and MS Data of the Berberine Derivatives **3**−**13**

						HR LSIMS	
compound	yield (%)	mp (dec) (°C)	c_R (min)	formula	LSIMS *m/z* $[M - Cl]^+$	calcd	found
3	75	206−207	15.03	$C_{21}H_{20}NO_4$	350	350.1391	350.1406
4	70	199−200	17.16	$C_{22}H_{22}NO_4$	364	364.1547	364.1557
5	60	186−187	18.75	$C_{23}H_{24}NO_4$	378	378.1704	378.1689
6	55	179−180	20.18	$C_{24}H_{26}NO_4$	392	392.1860	392.1868
7	50	208	18.57	$C_{26}H_{22}NO_4$	412	412.1548	412.1558
8	80	187−188	16.97	$C_{20}H_{17}NO_4Br$	414, 416	414.0340	414.0314
9	55	190−192	19.41	$C_{22}H_{21}NO_4Br$	442, 444	442.0652	442.0666
10	55	191−193	20.62	$C_{23}H_{23}NO_4Br$	456, 458	456.0809	456.0814
11	50	193−195	21.92	$C_{24}H_{25}NO_4Br$	470, 472	470.0966	470.0950
12	35	196−198	24.44	$C_{26}H_{29}NO_4Br$	498, 500	498.1278	498.1276
13	40	192−194	19.77	$C_{26}H_{21}NO_4Br$	490, 492	490.0652	490.0648

Table 3. *In Vitro* Antibacterial and Antifungal Activities of Berberine (**1**) and Its Derivatives (**3**−**13**)

	MIC (μg/mL)				
compound	*S. aureus*	*B. subtilis*	*S. enteritidis*	*E. coli* (IFO 026)	*C. albicans* (IFO 1061)
1	250[a]	1000[b]	500[b]	>2000[a]	500[a]
3	125	250	125	> 500	250
4	62.5	125	62.5	> 500	125
5	31.2	62.5	31.2	500	250
6	15.6	31.2	31.2	500	250
7	62.5	62.5	125	> 500	250
8	125	125	125	> 500	500
9	31.2	31.2	31.2	500	250
10	15.6	15.6	15.6	500	125
11	7.8	7.8	15.6	250	62.5
12	3.9	3.9	3.9	125	15.6
13	31.2	31.2	31.2	500	250
KA[c]	31.2	3.9	62.5	31.2	> 2000

[a] Previously reported data.[3] [b] Previously reported data.[4] [c] Kanamycin sulfate.

instrument. The secondary ion mass spectra (LSIMS) were measured using glycerol as matrix. HPLC analysis was performed on a Hitachi M-6200 Intelligent Pump (1 mL/ min) and Hitachi L-4000 UV detector (280 nm). Analyses were made on a Cosmosil $5C_{18}$-AR reversed-phase column (4.6 i.d. × 150 mm) eluting with 0.1M NH_4OAc (0.05%TFA)−MeOH (0.05% TFA), A/B, initial (25% of B), 10 min (50% of B), 20 min (80% of B).

Preparation of 8-alkyl- and 8-phenyl-berberine chlorides (3−7). Grignard reagents prepared from Mg turnings (3.8 g) and the corresponding alkyl and phenyl iodides (0.13 mol) in absolute ether (100 mL) were slowly added to the suspension of dry berberine chloride (0.03 mol) in absolute ether (100 mL) under N_2 at 0 °C. After 2 h of reflux, the 8-alkyl- and 8-phenyl-dihydroberberine iodides (**2** R = alkyl or phenyl) are obtained as usual and crystallized from 80% MeOH: 8-methyldihydro-berberine iodide [mp 244−245 °C (lit.[5] 249 °C)]; 8-ethyldihydroberberine iodide [mp 225−227 °C (lit.[6] 223 °C)]; 8-*n*-propyldihydroberberine iodide [mp 205−207 °C (lit.[6] 207 °C)]; 8-*n*-butyldihydroberberine iodide (mp 202−203 °C); 8-phenyldihydroberberine iodide (mp 219−221 °C). These hydroiodides (0.01 mol) in hot HOAc (100 mL) were treated with Br_2 (0.01mol) in HOAc (10 mL) under reflux for 1 h. After cooling, the precipitates were filtered and washed with 10% $Na_2S_2O_5$ solution, then with H_2O to yield the crude 8-alkyl- and 8-phenyl-berberine iodides, which were crystallized from 80% MeOH: 8-methylberberine iodide [mp 250−252 °C (dec) (lit.[6] 255−260 °C)]; 8-ethylberberine iodide [mp 242−243 °C (dec) (lit.[6] 248 °C)]; 8-*n*-propylberberine iodide [mp 243−245 °C (dec) (lit.[6] 246 °C)]; 8-*n*-butylberberine iodide [mp 245−246 °C (dec)]; 8-phenylberberine iodide [mp 248−250 °C (dec)]. These iodides were converted into yellow-orange crystalline chlorides either with AgCl in hot MeOH or using an Amberlite IRA-400 column: 8-methylberberine chloride (**3**) [mp 206−207 °C (dec)]; 8-ethylberberine chloride (**4**) [mp 199−200 °C (dec)]; 8-*n*-propylberberine chloride (**5**) (mp 186−187 °C); 8-*n*-butylberberine chloride (**6**) [mp 179−180 °C (dec)]; 8-phenylberberine chloride (**7**): mp 208 °C (dec).

For ^{1}H NMR and LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 2.

Preparation of 12-Bromoberberine Chloride (8). Reduction of **1** with $NaBH_4$ afforded **2** (R = H), mp 155−156 °C (lit.[7] 157−158 °C) as a base, which was converted into **2**-HCl, pale yellow crystals, mp177−178 °C by 3N HCl. Treatment **2**-HCl with 10 equiv of Br_2 and crystallization from 80% MeOH led to 12-bromoberberine (**8**), mp 187−188 °C. Bromination at C-12 follows from the ^{1}H NMR spectrum, which shows singlets only for the aromatic protons (Table 1). In the NOESY spectrum, 4-H and 5-H, 1-H and 13-H, 11-H and 10-OMe, and 8-H and 6-H show crosspeaks. For ^{1}H NMR and LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 2.

Preparation of 8-Alkyl-12-bromo- and 12-Bromo-8-phenyl-berberine Chlorides (9−13). These compounds were prepared by dehydrogenation of the pertinent **2**-iodides using 10 equiv of Br_2 (see above): 12-bromo-8-ethylberberine chloride (**9**) (mp 190−192 °C); 12-bromo-8-*n*-propylberberine chloride (**10**) (mp 191−193 °C); 12-bromo-8-*n*-butylberberine chloride (**11**) (mp 193−195 °C); 12-bromo-8-*n*-hexylberberine chloride (**12**) (mp 196−198 °C); and 12-bromo-8-phenylberberine chloride (**13**) (mp 192−194 °C). For ^{1}H NMR and LSIMS, HRLSIMS, and HPLC data, see Tables 1 and 2.

Microbiology. Antibacterial activity against *S. aureus*, *B. subtilis*, and *S. enteritidis*, isolated from hospitalized patients, and against *E. coli* (IFO 026) and antifungal activity against *C. albicans* (IFO 1061) were determined by means of the minimum inhibitory concentration (MIC) using the twofold serial broth dilution test in liquid nutrient medium and 24-well microplates. MIC was defined as the lowest concentration of the test substance that did not induce visible growth in comparison with a blank experiment.The substancs were dissolved in H_2O−1% DMF. Dilutions with the test medium furnished concentrations from 1 to 500 μg/mL. Blanks were prepared in H_2O−1% DMF only. Berberine (**1**) and KA were used as standard. The several 24-well plates, in which each well containined an appropriate growth medium with a different concentration of the respective berberine derivatives, were incubated with the test organism. The 24-well plate was incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for the fungus. Bacteria tested were preliminarily cultivated in 3% nutrient broth ('Nissui", Japan) at 37 °C, while *C. albicans* was cultivated in 3% malt extract powder ("Oriental", Japan) at 25 °C. All experiments were run in duplicate or triplicate. For measuring the growth of cells, a constant amount of sample (200 μL) was transferred to a 96-well test plate from individual wells (1 mL) of a 24-well plate. After incubation, the microbial growth was examined by measuring the optical density at 655 nm with a model 450 microplate reader (Bio-Rad).

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