New Stilbene Derivatives from *Calligonum leucocladum*

Mamoru Okasaka,[†] Yoshihisa Takaishi,*[,†] Kentarou Kogure,[†] Kenji Fukuzawa,[†] Hirohumi Shibata,[†] Tomihiko Higuti,[†] Gisho Honda,[‡] Michiho Ito,[‡] Olimjon K. Kodzhimatov,[§] and Ozodbek Ashurmetov[§]

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770-8505, Japan, Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida, Sakyo-ku, Kyoto, 606-8501, Japan, and Institute of Botany and Botanical Garden, F. Khodzhaev, st. 32, 700143, Tashkent, Uzbekistan

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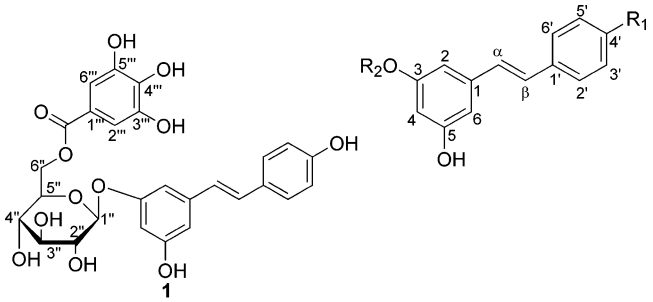
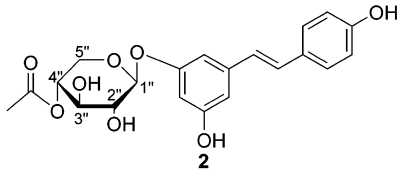
Two new stilbene derivatives, (*E*)-resveratrol 3-(6″-galloyl)-*O*-*β*-D-glucopyranoside (**1**) and (*E*)-resveratrol 3-(4″-acetyl)-*O*-*β*-D-xylopyranoside (**2**), and five known stilbene derivatives (**3**−**7**) were isolated from the dried aerial parts of *Calligonum leucocladum*. Their structures were established on the basis of spectroscopic evidence. Compound **1** showed antioxidant activity and a restorative effect of the inhibition of oxacillin to oxacillin/methicillin-resistant *Staphylococcus aureus*.

Calligonum leucocladum (Schrenk) Bunge belongs to the family Polygonaceae, with some 80 species distributed throughout Western Asia, Southern Europe, and North Africa. As a part of an ongoing study on Uzbekistan folk medicinal plants, we have investigated the constituents of *C. leucocladum*, of which the seeds are used for the treatment of syphilis and rheumatism and the leaves help promote a healthy gall bladder. *C. leucocladum* is also used for its tannins in dyeing and as a food plant.[1] Some phenolic compounds have been isolated in the underground parts of *C. leucocladum*.[2] We describe herein the isolation, structure elucidation, and activity in a radical-scavenging antioxidant test and in an antibacterial assay against oxacillin/methicillin-resistant *Staphylococcus aureus* (MRSA) of some stilbenoids isolated from the species.

The ethyl acetate extract and *n*-BuOH-soluble parts of the methanol extract of the dried aerial parts of *C. leucocladum* were separated by repeated column chromatography to give altogether 19 compounds, including two new stilbene derivatives (compounds **1** and **2**) and 17 known compounds [(*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside (**3**),[3] (*E*)-resveratrol 3-*O*-*β*-D-xylopyranoside (**4**),[4] pinosylvin (**5**),[5] pinosilvin 3-*O*-*β*-D-glucopyranoside (**6**),[6] (*E*)-resveratrol (**7**),[7] hexacosanoyl ferulate,[8] octacosanoyl ferulate,[9] *β*-sitosterone,[10] *β*-sitosterol,[10] oleanolic acid,[11] betulin,[12] pinocembrin,[13] pinocembrin 7-*O*-glucoside,[14] quercetin,[15] 5,7,3′-trihydroxyflavanone,[16] rhododendrin,[17] and salidroside[18]]. The known compounds exhibited physical and spectral data identical to values in the literature.

Compound **1** was assigned the molecular formula of $C_{27}H_{26}O_{12}$, as established from its negative-ion HRFABMS (*m*/*z* 541.1318). Its IR spectrum showed absorbances for a hydroxyl group (3370 cm^{-1}), an ester (1693 cm^{-1}), and aromatic groups (1604, 1511 cm^{-1}). The ^{1}H NMR spectrum of **1** showed the presence of nine aromatic protons [δ_H 7.30, 6.80 (each 2H, d, J = 8.8 Hz); 6.76, 6.69, 6.52 (each 1H, t, J = 1.6 Hz); 7.15 (2H, s)], two olefinic protons [δ_H 7.00, 6.78 (each 1H, d, J = 16.0 Hz)], and a sugar moiety. The ^{13}C NMR spectrum of **1** showed 27 carbon signals, including one carbonyl carbon, 18 aromatic carbons, two olefinic carbons, and a sugar moiety (Table 1). From the ^{1}H−^{1}H COSY spectrum and the above data, the presence of three





3: R_1 = OH, R_2 = β-D-glucopyranose
4: R_1 = OH, R_2 = β-D-xylopyranose
5: R_1 = H, R_2 = H
6: R_1 = H, R_2 = β-D-glucopyranose
7: R_1 = OH, R_2 = H

benzene rings was inferred, in the 1,4-disubstituted, 1,3,5-trisubstituted, and 1,3,4,5-tetrasubstituted forms. The presence of one *trans* double bond from the analysis of the HMBC spectrum was consistent with a stilbene moiety in compound **1**. From the coupling pattern of these two benzene rings and the presence of the sugar moiety, compound **1** was postulated as being a derivative of (*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside (**3**). The ^{13}C NMR spectrum of **1** was very similar to that of **3** except for one benzene ring, a carbonyl carbon, and one carbon of the sugar moiety. The structure of the remaining one benzene ring and carbonyl carbon was determined to be a galloyl ester from the molecular formula, the NMR data [δ_H 7.15 (2H, s), δ_C 110.2, 121.3, 139.9, 146.5, and 168.4], and the molecular formula. The remaining problem in the structural determination of **1** was the placement of the galloyl ester. The HMBC spectrum of **1** showed the following correlations: H-2‴ (H-6‴) with the carbonyl carbon (δ_C 168.4) and H_2-6″ with this same carbonyl carbon. Thus, the structure of **1** was elucidated as (*E*)-resveratrol 3-(6″-galloyl)-*O*-*β*-D-glucopyranoside.

Compound **2** was assigned the molecular formula $C_{21}H_{22}O_8$, as established from its negative-ion HRFABMS (*m*/*z* 401.1230). The IR spectrum showed hydroxyl (3390

* To whom correspondence should be addressed. Tel: 81-88-6337275. Fax: 81-88-6339501. E-mail: takaishi@ph.tokushima-u.ac.jp.
[†] University of Tokushima.
[‡] Kyoto University.
[§] Academy of Science Uzbekistan Institute of Botany.

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Table 1. NMR Spectral Data of Compounds **1** and **2**

	1[a]		**2**[a]	
C/H	δ_C[b]	δ_H (mult., *J* in Hz)[c]	δ_C[b]	δ_H (mult., *J* in Hz)[c]
1	141.5		141.4	
2	107.6	6.76 (t, 1.6)	107.3	6.75 (brs)
3	160.4		160.1	
4	104.3	6.52 (t, 1.6)	104.2	6.46 (brs)
5	159.4		159.6	
6	108.3	6.69 (t, 1.6)	108.4	6.68 (brs)
a	126.5	6.78 (d, 16.0)	126.6	6.88 (d, 16.3)
b	130.1	7.00 (d, 16.0)	130.0	7.04 (d, 16.3)
1′	130.2		130.3	
2′	128.9	7.30 (d, 8.8)	128.9	7.40 (d, 8.5)
3′	116.5	6.80 (d, 8.8)	116.5	6.81 (d, 8.5)
4′	158.3		158.5	
5′	116.5	6.80 (d, 8.8)	116.5	6.81 (d, 8.5)
6′	128.9	7.30 (d, 8.8)	128.9	7.40 (d, 8.5)
1″	102.5	4.97 (d, 7.2)	102.8	4.94 (d, 7.4)
2″	74.9	3.56 (m)	74.8	3.56 (dd, 7.4, 9.0)
3″	77.8	3.58 (m)	74.8	3.72 (t, 9.0)
4″	71.5	3.55 (m,)	73.0	4.83 (m)
5″	75.6	3.79 (m)	63.6	4.08 (dd, 5.4, 11.4)
				3.47 (dd, 9.9, 11.4)
6″	64.7	4.67 (dd, 2.0, 12.0)		
		4.47 (dd, 5.6, 12.0)		
1‴	121.3			
2‴	110.2	7.15 (s)		
3‴	146.5			
4‴	139.9			
5‴	146.5			
6‴	110.2	7.15 (s)		
−COO−	168.4			
−OAc			172.2	
			20.8	2.12 (s)

[a] CD_3OD. [b] 100 MHz. [c] 400 MHz.

cm^{-1}), carbonyl (1727 cm^{-1}), and aromatic group (1596, 1511 cm^{-1}) absorptions. The ^{1}H NMR spectrum of **2** showed the presence of seven aromatic protons [δ_H 7.40, 6.81 (each 2H, d, J = 8.5 Hz), 6.75, 6.68, 6.46 (each 1H, brs)], two olefinic protons [δ_H 7.04, 6.88 (each 1H, d, J = 16.3 Hz)], acetyl protons [δ_H 2.12 (3H, s)], and a sugar moiety. The ^{13}C NMR spectrum of **2** showed 21 carbon signals, including one carbonyl carbon, 12 aromatic carbons, two olefinic carbons, four methine carbons, one methylene carbon, and one methyl carbon (Table 1). These data were very similar to those of compound **4** except for the acetyl ester signals and one carbon of the sugar moiety. Comparing the ^{1}H NMR and ^{13}C NMR spectrum of both compounds **2** and **4**, H-3″ [2 (δ_H 3.72, t, J = 9.0 Hz); 4 (δ_H 3.50, m)], H-5″ [2 (δ_H 3.47, dd, J = 9.9, 11.4 Hz and 4.08, dd, J = 5.4, 11.4 Hz); 4 (δ_H 3.42, m and 3.98, dd, J = 5.2, 11.4 Hz)], and the C-3″ and C-5″ signals of **4** were shifted upfield and H-4″ [2 (δ_H 4.83, m); 4 (δ_H 3.64, m)] and the C-4″ signals of **4** were shifted downfield (Table 1). For this reason, it was determined that the acetyl group was connected to the xylose unit at C-4″. Compound **2** was elucidated, therefore, as (*E*)-resveratrol 3-(4″-acetyl)-*O*-*β*-D-xylopyranoside.

Interesting biological activities recently found for (*E*)-resveratrol derivatives include the inhibition of the growth of tumor cell lines in vitro, the inhibition of carcinogenesis in vivo,[19] and the induction of apoptosis[20] and suggest the importance of plants containing stilbenoids as potential resources for the development of new drugs. In the present study, *C. leucocladum* has been found to be a rich source of (*E*)-resveratrol derivatives.

The seven isolated stilbene derivatives (**1**−**7**) were evaluated for DPPH radical-scavenging activity.[21−24] The activities of **1** and **7** were more potent than that of L-cysteine, while the other compounds did not show activity in this assay (Table 2). When tested for activity against

Table 2. Effect of Compounds **1**−**7** on DPPH Radicals[a]

compound	ratio of absorbance (compound/L-cysteine)
1	1.30
2	0.20
3	0.54
4	0.42
5	0.52
6	0.14
7	1.77

[a] Data are expressed as the ratio of the absorbance of L-cysteine to each test compound (measurement at 517 nm; determination after 30 min; the concentrations of the test compounds and L-cysteine were 20 μM).

Table 3. Anti-MRSA Activities of Compounds **1**, **5**, and **7** and Oxacillin

	MIC (μg/mL)			
organism	oxacillin	**1**	**5**	**7**
MRSA 1	512	125	>250	>250
MRSA 2	256	125	>250	>250
MRSA 4	256	125	>250	>250
MRSA 5	256	>250	125	>250
MRSA 8	256	125	>250	>250
MRSA 12	512	250	>250	>250
MRSA COL	512	>250	125	>250

Table 4. Effects of Compounds **1** and **3** on MICs of Oxacillin against MRSA Strains

	oxacillin MIC (μg/mL)			
	1			**3**
organism	(12.5 μg/mL)	(25 μg/mL)	(50 μg/mL)	(100 μg/mL)
MRSA 1	512	256	64	512
MRSA 2	128	32	4	128
MRSA 4	256	128	32	128
MRSA 5	256	128	64	256
MRSA 8	128	32	8	128
MRSA 12	256	64	8	256
MRSA COL	512	256	64	256

MRSA,[25] compound **5** showed a minimum inhibitory concentration (MIC) of 125 μg/mL (Table 3). Although ineffective if tested alone, compound **1** restored the effectiveness of oxacillin against MRSA when the two compounds were used in combination (Table 4).[26−28] On comparing with compound **3**, the galloyl group of **1** may be considered to play a role in mediating this effect.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a DIP-370 digital polarimeter (JASCO). IR spectra were recorded on a FT-IR 420 Fourier transform infrared spectrometer (JASCO). NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both using TMS) spectra were measured on an AVANCE 400 Fourier transform spectrometer (Bruker), and MS were obtained on a JMSD-300 mass spectrometer (JEOL). Column chromatography: silica gel 60N (63-210 nm; Kanto Kagaku), Toyopearl HW-40 (Tosoh), Sephadex LH-20 (Pharmacia). HPLC columns: silica gel (Mightysil Si 60 250-20; Kanto Kagaku), gel permeation (H2001 and H2002; Shodex, GS-310 2G; Asahipak), ODS (Mightysil RP-18 GP 250-20; Kanto Kagaku).

Plant Material. The aerial parts of *Calligonum leucocladum* were collected from Kyzylkum, Uzbekistan, in April 2001. Herbarium specimens (EMS-01ky-16) are deposited in the herbarium of the Graduate School of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation. The dried aerial parts of *C. leucocladum* (740 g) were crushed and extracted successively

using a Soxhlet extractor with hexane, EtOAc, and MeOH. The hexane and EtOAc extracts were combined (22 g) and subjected to silica gel column chromatography (CC) with different solvents of increasing polarity (*n*-hexane−EtOAc; EtOAc−MeOH) to give 16 fractions (1−16). Fraction 2 (2.3 g) was separated using silica gel ($CHCl_3$−MeOH, 99:1, 95:5, 9:1, and MeOH) and gel permeation CC ($CHCl_3$) to give octacosanoyl ferulate (79 mg) and fraction 2.2. Fraction 2.2 was applied to silica gel CC ($CHCl_3$−MeOH, 99:1, 95:5, 9:1, and MeOH) to give *β*-sitosterone (9 mg). Fraction 3 (2.0 g) was separated using silica gel CC ($CHCl_3$−MeOH, 99:1, 95:5, 9:1, and MeOH), Toyopearl ($CHCl_3$−MeOH, 2:1), and GPC ($CHCl_3$) to give *β*-sitosterol (109 mg). Fraction 4 (1.0 g) was separated by silica gel CC ($CHCl_3$−MeOH, 99:1, 95:5, 9:1, and MeOH) to give three fractions (fractions 4.1−4.3). Fraction 4.1 was subjected to HPLC (silica gel, hexane−EtOAc) to give hexacosanoyl ferulate (6 mg). Fraction 4.2 was separated using Toyopearl ($CHCl_3$−MeOH, 2:1) and GPC ($CHCl_3$) to give betulin (5 mg) and pinocembrin (151 mg). Fraction 4.3 was subjected to GPC ($CHCl_3$) to give oleanolic acid (7 mg). Fraction 6 (627 mg) was subjected to GPC ($CHCl_3$) to give **5** (38 mg) and 5,7,3′-trihydroxyflavanone (3 mg). Fraction 9 (2.8 g) was washed with $CHCl_3$ to obtain the insoluble material as **7** (738 mg). Fraction 12 (1.0 g) was separated over Toyopearl ($CHCl_3$−MeOH, 1:1) to give two fractions (fractions 12.1 and 12.2). Fraction 12.1 was applied to silica gel CC ($CHCl_3$−MeOH, 8:2, and MeOH) to give quercetin (9 mg). Fraction 12.2 was separated using GPC (MeOH) and silica gel CC ($CHCl_3$−MeOH, 8:2, and MeOH) to give **2** (24 mg). Fraction 13 (7.2 g) was applied to Sephadex LH-20 (MeOH) to give six fractions (fractions 13.1−13.6). Fraction 13.1 was separated by silica gel CC ($CHCl_3$−MeOH, 9:1, 8:2, and MeOH) and GPC (MeOH) to give **3** (152 mg) and **4** (263 mg). Fraction 13.2 was applied to silica CC ($CHCl_3$−MeOH, 8:2, 7:3, and MeOH) to give rhododendrin (20 mg). Fraction 13.3 was subjected to silica gel CC ($CHCl_3$−MeOH, 7:3, and MeOH) to give pinocembrin 7-*O*-glucoside (114 mg). Fraction 13.4 was separated by silica gel CC ($CHCl_3$−MeOH, 8:2, 7:3, and MeOH) and GPC (MeOH) to give **1** (16 mg). Fraction 13.5 was subjected to GPC (MeOH) to give salidroside (31 mg). Fraction 13.6 was separated by silica gel CC ($CHCl_3$−MeOH, 8:2, 7:3, and MeOH) and GPC (MeOH) to give **6** (30 mg).

(*E*)-Resveratrol 3-(6″-galloyl)-*O*-*β*-D-glucopyranoside (1): brown amorphous powder; $[\alpha]_D$ −89.2° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3370, 1693, 1604, 1511, 1450, 1334, 1234, 1068 cm^{-1}; ^1H NMR (CD_3OD) data, see Table 1; ^{13}C NMR (CD_3OD) data, see Table 1; HRFABMS *m*/*z* 541.1318 $[M - H]^-$ (calcd for $C_{27}H_{25}O_{12}$, 541.1346).

(*E*)-Resveratrol 3-(4″-acetyl)-*O*-*β*-D-xylopyranoside (2): brown amorphous powder; $[\alpha]_D$ −62.8° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3390, 2923, 1727, 1596, 1511, 1446, 1365, 1253, 1164 cm^{-1}; ^1H NMR (CD_3OD) data, see Table 1; ^{13}C NMR (CD_3OD) data, see Table 1; HRFABMS *m*/*z* 401.1230 $[M - H]^-$ (calcd for $C_{21}H_{21}O_8$, 401.1236).

Radical-Scavenging Assay. The radical-scavenging activity of test compounds (20 *μ*M) in ethanolic solution was determined from the decrease in the absorbance of DPPH radicals at 517 nm due to their scavenging of an unpaired electron of the stable DPPH radicals in a mixture of 10 mL of ethanol, 30 mL of 50 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 5.5), and 10 mL of 0.5 mM DPPH in ethanol. L-Cysteine was used as reference compound.

Bacterial Strains. *Staphylococcus aureus* strains 1, 2, 4, 5, 8, and 12 (clinical isolates) and strain COL were kindly supplied by the late Dr. Toru Usui (Kyoto Microbiological Institute, Kyoto, Japan) and Dr. John J. Iandolo (University of Oklahoma, Health Sciences Center, Tulsa, OK), respectively.

Antibacterial Activity. The MICs of test compounds and oxacillin, both alone and in combination with 12.5, 25, and 50 *μ*g/mL of test compound, were determined by the 2-fold plate dilution method, with BBL Mueller-Hinton II agar (Becton, Dickinson and Co., Franklin Lakes, NJ) supplemented with 25 *μ*g/mL of Ca^{2+}, 50 *μ*g/mL of Mg^{2+}, and 2% NaCl. Overnight cultures of test strains at 37 °C in Mueller-Hinton broth (Becton, Dickinson and Co.) were diluted with 0.85% NaCl, and the bacteria (about 10^6 cfu/mL) were applied by an inoculator onto the surfaces of 10 mL agar layers containing test compounds or oxacillin in the presence or absence of 12.5, 25, and 50 *μ*g/mL of the test compound. The plates were incubated at 37 °C for 24 h and then read. MIC values were recorded as the lowest concentration of the test compound that completely inhibited growth.

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