New Paeonilactone-A Adducts Formed by Anaerobic Incubation of Paeoniflorin with *Lactobacillus brevis* in the Presence of Arylthiols

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Received January 11, 2001; accepted March 21, 2001

During the course of preparing anticonvulsant paeonimetabolin-I adducts, new paeonilactone-A adducts: 9-phenylthiopaeonilactone-A, 9-(*o*-tolylthio)paeonilactone-A, 9-(*m*-tolylthio)paeonilactone-A, 9-(*p*-tolylthio)-paeonilactone-A and 9-(2-naphthylthio)paeonilactone-A, were obtained along with expected paeonimetabolin-I adducts by anaerobic incubation of paeoniflorin from peony roots with *Lactobacillus brevis* in the presence of the aromatic thiols, phenylthiol, *o*-tolylthiol, *m*-tolylthiol, *p*-tolylthiol and 2-naphthylthiol. The structures of these compounds were determined by spectroscopic methods including two dimensional (2D) NMR.

Key words paeonilactone-A; paeoniflorin; intestinal bacteria; *Lactobacillus brevis*

A series of metabolites termed paeonimetabolins-I (**3a**), -II and -III were reportedly obtained by anaerobic incubation of paeoniflorin (**1**), a major monoterpene glycoside from peony roots, with human intestinal bacteria.[1,2] In the presence of alkylthiols and arylthiols, various paeonimetabolin-I adducts were also obtained after incubation of **1** with *Lactobacillus brevis*,[3—5] in which thiol groups were covalently linked at the C-8 position of **3a**. Hattori *et al*., in contrast, reported that albiflorin (**2**), a minor component of peony roots, was transformed to paeonilactone-A (**4a**) and paeonilactone-B, when incubated with human intestinal bacteria.[6] Both metabolites are isolated from fresh peony roots.[7]

In the course of our studies on the transformation of common natural products to biologically active compounds using human intestinal bacterial, we recently prepared a number of paeonimetabolin-I derivatives from **1**, in which some of these compounds had potent anticonvulsant activity.[5] In the present paper, we describe new compounds characterized as paeonilactone-A adducts obtained as minor metabolites in the transformation of **1** with *L. brevis* in the presence of various aromatic thiols.

Results and Discussion

After anaerobic incubation of **1** with *L. brevis* in the presence of various aromatic thiols, the metabolites were separated and purified by repeated column chromatography to give minor compounds **4b**—**f**, together with the expected major paeonimetabolin-I adducts.[5] The IR spectra of **4b**—**f** revealed absorption bands at 3400 (OH), 1720—1725 (cyclohexyl C=O) and 1760—1790 (γ-lactone C=O) cm^{-1}. The ^{1}H-NMR spectra showed that all proton signals attached to the main skeleton of these adducts could be assigned by comparison with the reported spectral data of **4a**.[7] No appreciable deviation of the chemical shifts of the respective protons of **4b**—**f** was observed except H-9 ($\Delta\delta$ *ca.* +2 ppm). In addition, the ^{13}C-NMR spectral data of **4b**—**f** (Table 1) were quite similar to those of **4a**, but showed downfield shifts of C-9 ($\Delta\delta$ *ca.* 22 ppm), C-8 ($\Delta\delta$ *ca.* 5 ppm) and C-4 ($\Delta\delta$ *ca.* 3 ppm) signals, when compared to the corresponding ones of **4a**. The spectra also revealed signals due to aromatic carbon atoms. The structures of these adducts **4b**—**f** were further confirmed by proton–proton homonuclear correlation spectroscopy (^{1}H–^{1}H COSY) and ^{1}H–^{13}C COSY experiments.

In the ^{1}H–^{1}H COSY spectrum of **4e**, signals at δ 1.90 (H-2β) and 2.55 (H-2α) were correlated with each other, and also with a signal at δ 4.90 (H-3), which was subsequently correlated with a signal at δ 3.16 (H-4). Methylene protons at δ 2.85 and 2.76 (H-5β, H-5α) were coupled to each other, and correlated with a signal at δ 3.16 (H-4), which were correlated with signals at δ 2.60, 2.76, 2.85 and 4.90 (H-7, H-5α, H-5β and H-3, respectively). The stereochemistry of **4e** was established by a nuclear Overhouser effect spectroscopy (NOESY) experiment. In this NOESY experiment, NOE correlations were observed between H_3-10 and H-2α, H-3, H-5β, between H-3 and H-2α, H-4, H-5α and between H-7 and H-2β, H-4. Comparison of the NOESY spectrum of **4e** with the reported data of **4a** indicated that the stereostructure of **4e** was identical with that of **4a**.[7]

In the absence of either intestinal bacteria or aromatic thiols, no adducts were formed, suggesting that the reaction is initiated by bacterial hydrolysis of a sugar moiety in **1**. Furthermore, it is of interest that paeonilactone-A adducts were not formed with aliphatic thiols but with aromatic thiols, al-

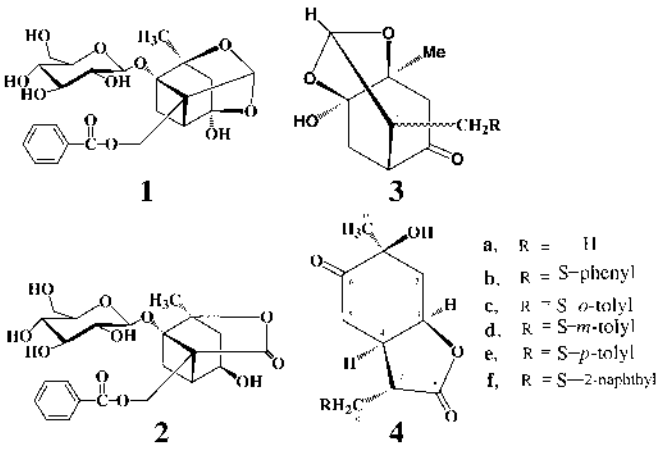

Chart 1. Structures of Paeoniflorin (**1**), Albiflorin (**2**), Paeonimetabolin-I (**3a**) and Its Adducts (**3b**—**f**), and Paeonilactone-A (**4a**) and Its Adducts (**4b**—**f**)

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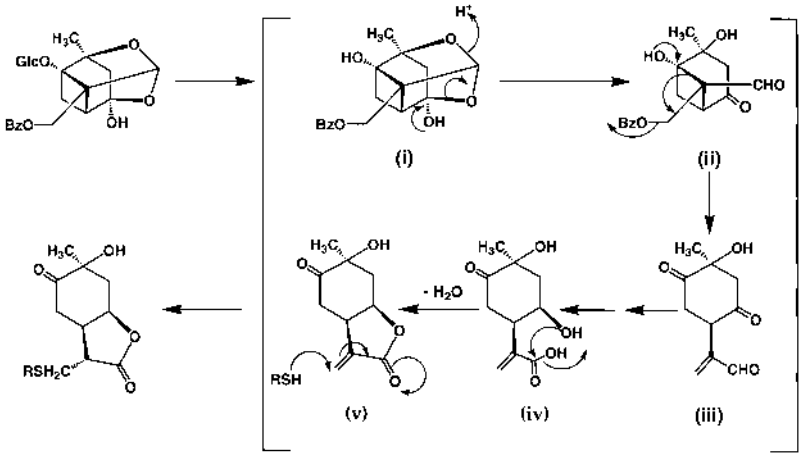

Chart 2. Possible Processes for the Formation of Paeonilactone-A Adducts (**4b**—**f**)

Table 1. ^{13}C-NMR Spectral Data ($CDCl_3$) of Paeonilactone-A and Its Adducts (**4a**—**f**)

Carbon No	**4a**[a,b]	**4b**[a,c]	**4c**[d]	**4d**[d]	**4e**[d]	**4f**[c]
1	73.5	73.5	73.5	72.1	73.2	73.4
2	42.4	42.0	42.0	42.2	41.7	42.0
3	73.6	74.1	74.1	73.9	73.8	74.1
4	37.9	41.0	41.0	41.7	40.6	41.0
5	35.6	36.7	36.7	36.4	36.4	36.7
6	210.6	210.4	210.4	210.0	210.6	210.4
7	44.2	43.7	43.8	43.4	43.4	43.7
8	170.0	175.1	175.1	175.2	175.2	175.2
9	13.3	34.3	33.0	33.9	34.7	34.0
10	25.0	25.3	25.3	25.0	25.6	25.2
Thiol residue		Phenyl residue	*o*-Tolyl residue	*m*-Tolyl residue	*p*-Tolyl residue	2-Naphthyl residue
1		134.4	138.6	139.0	137.6	129.2
2		129.4	129.4	127.9	130.5	133.7
3		130.5	137.0	134.3	131.1	127.8
4		128.4	127.1	127.6	130.1	128.7
4a						128.4
5			130.7	131.4		127.4
6			126.8	129.0		126.4
7			20.8	21.0	21.1	127.0
8						127.8
8a						132.2

a) Reported.[4] *b*) 75 MHz. *c*) 100 MHz. *d*) 125 MHz.

though both types of thiols resulted in the formation of paeonimetabolin-I adducts as major products.

Chart 2 shows the possible processes for the formation of paeonilactone-A adducts **4b**—**f**. Paeoniflorin (**1**) converts to an aglyone i by bacterial β-glucosidase, which subsequently leads to iii by cleavage of a four-membered ring and debenzoylation. This intermediate iii may be converted to v through iv, possibly by redox reaction followed by lactonization. Michael addition of aromatic thiols on the double bond conjugated with a carbonyl group of v leads to the formation of **4b**—**f**. In terms of steric and conformational factors, α-orientation of the arylthio residues may be explained.

Since paeonimetabolin-I adducts possessing various alkylthio and arylthio groups prepared by intestinal bacterial transformation showed more potent anticonvulsant activity than paeonimetabolin-I (**3a**) itself as reported in previous papers,[5] it is worthwhile examining anticonvulsant activity of the new adducts as well as other biological activity, in comparison with those of paeonilactone A (**4a**) present in the peony roots.

Experimental

Instruments Melting points were determined on a micromelting point apparatus (L-272, Yanaco, Japan) and are uncorrected. IR spectra were measured with a JASCO FT/IR-230 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured with a Varian GEMINI 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz), a JEOL-JNM-GX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Varian UNITY 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrophotometer and all chemical shifts are given in ppm relative to tetramethylsilane (TMS). ^{1}H–^{1}H COSY, heteronuclear multiple quantum coherence (HMQC) and NOESY experiments were performed with the usual pulse sequence and data processing was obtained with standard Varian software. Electron impact (EI) and high resolution (HR) MS were measured with a JEOL JMS-AX 505 spectrometer at an ionization voltage of 70 eV.

Chromatography Thin-layer chromatography was carried out on precoated Silica gel 60 F_{254} plates (0.25 mm thickness, Merck, Darmstadt, Germany) and spots were detected under UV light or after spraying with anisaldehyde–H_2SO_4 reagent followed by heating. Silica gel 60 (70—230 mesh, Merck) and Diaion HP-20 (Ion Exchange Resin, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Analytical (HPLC) was performed on a CCPM-II (Tosoh, Tokyo, Japan) equipped with a Tosoh UV-8020 spectrometer and a Shimadzu C-R 6A chromatopac

(Shimadzu Co. Ltd, Kyoto, Japan) utilizing a Tosoh ODS-80Ts column [4.6 (i.d.)×150 mm]. The flow rate was kept at 1.0 ml/min and the peaks were monitored at 254 nm using the following solvent system: A=a buffer solution consists of 50 mM KH_2PO_4 and 0.1% H_3PO_4 and CH_3CN (95 : 5); B=H_2O : CH_3CN (20 : 80).

Chemicals Paeoniflorin (**1**) was isolated from the dried roots of *Paeonia lactiflora* PALL according to the method of Kaneda *et al.*[8] and its purity (99.9%) was determined by HPLC under the abovementioned conditions. General anaerobic medium (GAM) was a product of Nissui Seiyaku Co., Ltd., Tokyo, Japan. All chemicals used were of analytical grade.

Formation of Paeonilactone-A Adducts from 1 Paeonilactone-A adducts (**4b**—**f**) were obtained as minor metabolites by anaerobic incubation of **1** with a human intestinal bacterium in the presence of various arylthiols. Briefly, a precultured bacterial suspension (600 ml) of *L. brevis* was added to GAM broth (6 l) and anaerobically cultured for 18 h at 37 °C. The culture was centrifuged at 8000×***g*** for 10 min. The precipitates formed were washed with a saline solution, and suspended in 50 mM phosphate buffer (pH 7.3, 900 ml). An arylthiol (5.0 mmol in 5 ml of water or methanol), such as phenyl-, *o*-tolyl-, *m*-tolyl-, *p*-tolyl- or 2-naphthylthiol, and **1** (1.2 g, 2.5 mmol in 10 ml buffer solution) were added to each of the bacterial suspensions. The mixture was anaerobically incubated for 6 h at 37 °C, then passed through a Diaion column and washed with distilled water (3 l). The column was eluted with methanol (2 l), and the eluate was evaporated *in vacuo* to give a residue (0.3—0.6 g). The residue was repeatedly chromatographed on a column of silica gel with benzene : acetone (9 : 1) to give paeonimetabolin-I adducts and paeonilactone-A adducts. In the presence of phenyl-, *o*-tolyl-, *m*-tolyl-, *p*-tolyl- and 2-naphthylthiols, the respective adducts were obtained in the following yields (percentages were calculated from a starting material of **1**): 8-phenylthiopaeonimetabolin-I (**3b**, 230 mg, 30%) and 9-phenylthiopaeonilactone-A (**4b**, 8 mg, 1.0%); 8-(*o*-tolylthio)paeonimetabolin-I (**3c**, 185 mg, 23%) and 9-(*o*-tolylthio)paeonilactone-A (**4c**, 25 mg, 3.1%); 8-(*m*-tolylthio)paeonimetabolin-I (**3d**, 180 mg, 22.5%) and 9-(*m*-tolylthio)paeonilactone-A (**4d**, 25 mg, 2.8%); 8-(*p*-tolylthio)paeonimetabolin-I (**3e**, 190 mg, 24%) and 9-(*p*-tolylthio)paeonilactone-A (**4e**, 20 mg, 2.5%); 8-(2-naphthylthio)paeonimetabolin-I (**3f**, 205 mg, 23%) and 9-(2-naphthylthio)paeonilactone-A (**4f**, 42 mg, 4.7%).

9-Phenylthiopaeonilactone-A (**4b**): White solid substance. IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1790 (γ-lactone C=O), 1720 (cyclohexyl C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.90 (1H, dd, J=14.3, 10.4 Hz, H-2β), 2.55 (1H, dd, J=14.6, 6.6 Hz, H-2α), 4.90 (1H, ddd, J=10.4, 7.7, 6.6 Hz, H-3), 3.16 (1H, m, H-4), 2.73 (1H, dd, J=15.4, 7.1 Hz, H-5α), 2.80 (1H, dd, J=16.0, 3.5 Hz, H-5β), 2.63 (1H, ddd, J=10.4, 7.7, 3.8 Hz, H-7), 3.10 (1H, dd, J=13.7, 7.7 Hz, H-9), 3.50 (1H, dd, J=14.3, 4.4 Hz, H-9), 1.41 (3H, s, H-10), 3.60 (1H, br s, OH), phenyl residue: 7.2—7.4 (5H, m, aromatic protons).^{13}C-NMR see Table 1. EI-MS: 306 $[M]^+$, 180, 123, 110, 43, 18. HR-EI-MS: Found 306.0955, Calcd for $[M]^+$ $C_{16}H_{18}O_4S$: 306.0984.

9-(*o*-Tolylthio)paeonilactone-A (**4c**): White solid substance. IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1780 (γ-lactone C=O), 1720 (cyclohexyl C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.93 (1H, dd, J=14.3, 9.9 Hz, H-2β), 2.55 (1H, dd, J=14.6, 6.0 Hz, H-2α), 4.90 (1H, ddd, J=9.9, 7.7, 6.0 Hz, H-3), 3.16 (1H, m, H-4), 2.69 (1H, dd, J=15.8, 7.4 Hz, H-5α), 2.82 (1H, dd, J=16.2, 3.4 Hz, H-5β), 2.66 (1H, ddd, J=9.9, 7.7, 3.8 Hz, H-7), 3.10 (1H, dd, J=13.7, 7.7 Hz, H-9), 3.44 (1H, dd, J=13.7, 3.8 Hz, H-9), 1.42 (3H, s, H-10), 3.60 (1H, br s, OH), *o*-tolyl residue: 7.2—7.4 (4H, m, aromatic protons), 2.4 (3H, s, H-7). ^{13}C-NMR: see Table 1. EI-MS: 320 $[M]^+$, 194, 137, 124, 91, 55, 43, 18. HR-EI-MS: Found 320.1051, Calcd for $[M]^+$ $C_{17}H_{20}O_4S$: 320.1082.

9-(*m*-Tolylthio)paeonilactone-A (**4d**): White solid substance. IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1780 (γ-lactone C=O), 1720 (cyclohexyl C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.90 (1H, dd, J=14.0, 10.0 Hz, H-2β), 2.55 (1H, dd, J=14.0, 5.3 Hz, H-2α), 4.90 (1H, ddd, J=10.2, 7.7, 6.2 Hz, H-3), 3.16 (1H, m, H-4), 2.76 (1H, dd, J=15.2, 7.7 Hz, H-5α), 2.86 (1H, dd, J=16.2, 3.4 Hz, H-5β), 2.61 (1H, ddd, J=10.0, 8.0, 3.8 Hz, H-7), 3.08 (1H, dd, J=14.0, 6.0 Hz, H-9), 3.48 (1H, dd, J=15.0, 4.0 Hz, H-9), 1.41 (3H, s, H-10), 3.60 (1H, br s, OH), *m*-tolyl residue: 7.0—7.2 (4H, m, aromatic protons), 2.4 (3H, s, H-7). ^{13}C-NMR: see Table 1. EI-MS: 320 $[M]^+$, 194, 137, 124, 91, 55. HR-EI-MS: Found 320.1107, Calcd for $[M]^+$ $C_{17}H_{20}O_4S$: 320.1082.

9-(*p*-Tolylthio)paeonilactone-A (**4e**): White solid substance. IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1780 (γ-lactone C=O), 1720 (cyclohexyl C=O). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.90 (1H, dd, J=14.0, 10.0 Hz, H-2β), 2.55 (1H, dd, J=14.0, 6.0 Hz, H-2α), 4.90 (1H, ddd, J=10.0, 8.0, 6.0 Hz, H-3), 3.16 (1H, m, H-4), 2.76 (1H, dd, J=16.4, 7.5 Hz, H-5α), 2.85 (1H, dd, J=16.4, 3.4 Hz, H-5β), 2.60 (1H, ddd, J=10.0, 8.0, 6.0 Hz, H-7), 3.10 (1H, dd, J=14.0, 6.0 Hz, H-9), 3.4 (1H, dd, J=14.0, 4.0 Hz, H-9), 1.41 (3H, s, H-10), 3.60 (1H, br s, OH), *p*-tolyl residue: 7.13 (2H, dd, J=8.3, 0.4 Hz, H-2 and H-6), 7.31 (2H, dd, J=6.4, 1.9 Hz, H-3, H-5), 2.30 (3H, s, H-7). ^{13}C-NMR: see Table 1. EI-MS: 320 $[M]^+$, 194, 137, 124, 91, 55. HR-EI-MS: Found 320.1063, Calcd for $[M]^+$ $C_{17}H_{20}O_4S$: 320.1082.

9-(2-Naphthylthio)paeonilactone-A (**4f**): White solid substance. IR (KBr) ν_{max} cm^{-1}: 3300 (OH), 1760 (γ-lactone C=O), 1725 (cyclohexyl C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.89 (1H, dd, J=14.0, 10.0 Hz, H-2β), 2.51 (1H, dd, J=14.0, 5.5 Hz, H-2α), 4.88 (1H, ddd, J=10.0, 8.0, 6.5 Hz, H-3), 3.16 (1H, m, H-4), 2.76 (1H, dd, J=16.5, 7.7 Hz, H-5α), 2.86 (1H, dd, J=16.5, 3.3 Hz, H-5β), 2.66 (1H, ddd, J=11.0, 8.0, 4.0 Hz, H-7), 3.20 (1H, dd, J=14.7, 7.7 Hz, H-9), 3.59 (1H, dd, J=14.0, 4.0 Hz, H-9), 1.42 (3H, s, H-10), 3.59 (1H, br s, OH), naphthyl residue: 7.4—7.8 (7H, m, naphthyl protons). ^{13}C-NMR: see Table 1. EI-MS: 356 $[M]^+$, 160, 128, 115, 108. HR-EI-MS: Found 356.1076, Calcd for $[M]^+$ $C_{20}H_{20}O_4S$: 356.1082.

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