Potent Anticonvulsant Paeonimetabolin-I Derivatives Obtained by Incubation of Paeoniflorin and Thiol Compounds with *Lactobacillus brevis*

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Seventeen thiopaeonimetabolin-I adducts were obtained as mixtures of diastereoisomers after incubation of paeoniflorin with *Lactobacillus brevis* in the presence of various thiols. Four compounds, 8-(*n*-hexylthio)- (8), 8-cyclopentylthio-, 8-(*p*-tolyl)thio- and 8-benzoylthio- (18) paeonimetabolins, showed 100% protection against pentylenetetrazole-induced convulsions at doses of 0.125, 0.25, or 0.50 mmol/kg, relative to valproic acid (100% protection at 1.5 mmol/kg). For 8 and 18, the principle anticonvulsant activity resided in the (7*S*)-isomers while (7*R*)-isomers showed muscle relaxation effects.

Key words paeoniflorin; thiopaeonimetabolin-I adduct; *Lactobacillus brevis*; anticonvulsant activity

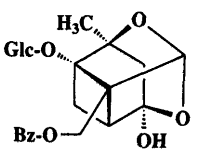
Hattori *et al.* reported that paeoniflorin (**1**, the major monoterpene glycoside from peony root) was transformed into a series of metabolites by human intestinal bacteria,[1,2] and that paeonimetabolin-I (**2**), its major metabolite, showed anticonvulsant activity in EI mice, a model animal of heredity epilepsy.[3] Thiol adducts of **2** were also obtained from **1** after incubation with *Lactobacillus brevis*, a human intestinal bacteria, in the presence of various thiols.[4] These adducts possessed similar structures in which the thiol residues are covalently bonded at the C-8 position of **2** through sulfide linkages, and seem to be more lipophilic than **2**.

To search for new drugs with selective anticonvulsant activity and less toxicity, a number of aliphatic and aromatic thiols were chosen to increase the anticonvulsant activity of **2**, adopting the method reported by one of us.[4] Accordingly, 17 thiopaeonimetabolin-I adducts (**3**—**19**) were obtained by incubation of **1** with *L. brevis* in the presence of various thiols (for 6 h in 0.05 M K-phosphate buffer, pH 7.3). The products (in yields of 20—30%) were purified by filtration through a column of Diaion HP-20 (eluted with H_2O followed by MeOH), and the MeOH eluate was chromatographed on silica gel with C_6H_6 and C_6H_6–Me_2CO.

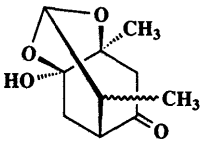
The structures of **3**—**19** were assigned on the bases of their EI-MS, IR, and NMR spectral data. The EI-MS spectra of these compounds showed molecular ion peaks which were consistent with their respective molecular formulas, and all spectra displayed the most common fragment ion peak at *m/z* 197 $[M-SR]^+$. Absorption bands at 3400 cm^{-1} (OH) and 1720—1730 (C=O) were seen in the IR spectra of all compounds. When compared with that reported for **2**, most of signals assigned for the cage-like skeleton remain essentially unaffected in **3**—**19**. However, the ^{1}H-NMR spectra of **3**—**19** showed paired singlets at δ_H 5.13—5.55 (in **2**, at δ_H 5.14—5.17) characteristic for H-9 of the diastereoisomeric mixtures obtained (with preference for the 7*S* isomers).

The ^{13}C-NMR spectra of **3**—**19** are comparable with that of **2**, except that the thiol residues induced a downfield shift of C-8 by *ca.* 15—17 ppm. From these findings, it is evident that **3**—**19** are the thiol adducts of **2**.

The anticonvulsant activity of **3**—**19** was preliminarily investigated in male ddY mice with a body weight of 30—35 g using the subcutaneous pentylenetetrazol (PTZ) seizure threshold test[5] and sodium valproate (1.5 mmol/kg) as a positive control. Each compound at doses of 0.125, 0.25, and 0.50 mmol/kg (suspended in 0.5% Tween 80 in saline) was injected intraperitoneally in a group of 6 animals. Thirty minutes later, PTZ (100 mg/kg in saline) was injected subcutaneously in a loose fold of skin on the back of the neck. The clonic and tonic convulsions and then death were observed for 1 h. Protection was defined as the ability of the compound to prevent threshold seizures (single 5-sec episode of clonic spasms).[6] From this experiment, it was apparent that **3**—**19** possessed more potent anticonvulsant activity than **2**. Of these, 13 compounds (**3**—**8**, **10**, **12**—**16**, and **18**) showed dose-dependent protection against clonic and tonic convulsions. Complete protection against convulsions was effectively demonstrated by **8** (at a dose of 0.125 mmol/kg) and **18** (at 0.250 mmol/kg), while 100% protection of **10** and **15** was only achieved at 0.500 mmol/kg body weight (Table 1). Interestingly, the 7*S* isomers of **8** and **18** ($[\alpha]_D$ −2.8° and −22.7°, respectively, in $CHCl_3$), obtained by medium pressure liquid chromatography were found to be responsible for the anticonvulsant activity rather than the 7*R* ones ($[\alpha]_D$ +3.5° and −49°, respectively) which showed muscle relaxation effects. No compound showed any appreciable toxicity within the tested doses using the acute toxicity test and grip test.



1



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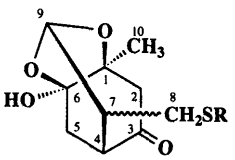

3, R = *n*-propyl
4, R = *iso*-propyl
5, R = *n*-butyl
6, R = *sec*-butyl
7, R = *iso*-butyl
8, R = *n*-hexyl
9, R = allyl
10, R = cyclopentyl
11, R = cyclohexyl
12, R = phenyl
13, R = *o*-tolyl
14, R = *m*-tolyl
15, R = *p*-tolyl
16, R = 2-naphthyl
17, R = benzyl
18, R = benzoyl
19, R = acetyl

Chart 1

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Table 1. Effects of Paeonimetabolin-I Adducts (**8**, **10**, **15** and **18**) on Clonic and Tonic Convulsions

Compd. No.	Dose (mmol/kg)	Latency (min±S.E.)		% inhibition	
		Clonic convulsions	Tonic convulsions	Clonic convulsions	Tonic convulsions
Control		5—9	16—20	0	0
2[a]	0.500	17.2±2.4**	21.2±2.7*	0	0
	0.250	7.8±0.8	9.3±2.0	0	0
	0.125	8.5±1.0	10.2±0.9	0	0
8[a]	0.500	>60.0***	>60.0***	100	100
	0.250	>60.0***	>60.0***	100	100
	0.125	>60.0***	>60.0***	100	100
10[a]	0.500	>60.0***	>60.0***	100	100
	0.250	31.8±9.0**	38.0±7.5***	33	33
	0.125	10.2±1.1	16.0±1.3	0	0
15[a]	0.500	>60.0***	>60.0***	100	100
	0.250	45.0±9.5*	49.5±6.9**	67	67
	0.125	28.2±10.2	31.0±9.2	33	33
18[a]	0.500	>60.0***	>60.0***	100	100
	0.250	>60.0***	>60.0***	100	100
	0.125	46.0±8.9***	48.67±5.4***	67	50
8 (7*S*)	0.125	>60.0***	>60.0***	100	100
	0.100	49.5±6.7***	51.7±5.4***	67	67
8 (7*R*)	0.500	10.7±1.8	17.3±2.9	0	0
	0.250	12.0±1.6	10.2±0.9	0	0
18 (7*S*)	0.250	>60.0***	>60.0***	100	100
	0.125	49.2±7.1***	49.2±7.1***	100	100
18 (7*R*)	0.250	—[b]	—[b]	—[b]	—[b]

a) The ratios of the two diastereoisomers were 1 : 2, 1 : 1, 1 : 1, 1 : 1, 1 : 3 for **2**, **8**, **10**, **15**, and **18**, respectively. *b*) Complete muscle relaxation was observed by the grip test. Statistical significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The present findings suggest that introduction of thiol residues at C-8 of **2** markedly enhanced its anticonvulsant activity, and that the 7*S* isomers (*vs.* the counterparts) of these derivatives were responsible for such activity. Further pharmacological and toxicological studies on these compounds are currently being conducted in our laboratories.

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