Protective Effect of Sodium L-Malate, an Active Constituent Isolated from Angelicae Radix, on *cis*-Diamminedichloroplatinum(II)-Induced Toxic Side Effect

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The effects of ingredients of Shi-Quan-Da-Bu-Tang (Juzen-taiho-to) on the nephrotoxicity and bone marrow toxicity caused by i.p. administration of 3 mg/kg *cis*-diamminedichloroplatinum (II) (CDDP) 9 times (on days 3, 4, 5, 6, 7, 8, 10, 11, 12) were examined in ddY mice s.c. inoculated with sarcoma 180 (S-180) cells on day 1. Angelicae Radix showed the strongest protective effect against the toxicity among the ingredients. The ED_{50} of a water extract of Angelicae Radix was 17.8 mg/kg for nephrotoxicity (indicated by an increase in blood urea nitrogen) and 59.4 mg/kg for bone marrow toxicity (indicated by a decrease in white blood cell count), when it was administered perorally (*p.o.*) on days 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15. The water extract did not exert any significant effect on the antitumor activity of CDDP. Bioassay-directed fractionation of the water extract resulted in isolation of a constituent having protective effects against the toxicity: sodium L-malate, $C_4H_4Na_2O_5$, was found to exhibit protective effects against both nephrotoxicity (ED_{50}: 0.4 mg/kg, *p.o.*) and bone marrow toxicity (ED_{50}: 1.8 mg/kg, *p.o.*), without reducing the antitumor activity of CDDP. These findings indicate that Angelicae Radix and its constituent sodium L-malate could provide significant protection against CDDP-induced nephrotoxicity and bone marrow toxicity without reducing the antitumor activity.

Keywords cisplatin; nephrotoxicity; Angelicae Radix; sodium malate; Juzen-taiho-to; bone marrow toxicity

cis-Diamminedichloroplatinum (II) (CDDP) is one of the most active antitumor drugs and is used to treat a variety of malignancies.[1,2] However, the clinical use of CDDP is limited by its severe side effects, such as nephrotoxicity.[3–5] The herbal medicine Shi-Quan-Da-Bu-Tang (Juzen-taiho-to) consists of 10 ingredients, *i.e.*, Angelicae Radix (3 g), Hoelen (3 g), Glycyrrhizae Radix (2 g), Ginseng Radix (3 g), Astragali Radix (3 g), Cinnamomi Cortex (3 g), Atractylodis Rhizoma (3 g), Paeoniae Radix (3 g), Cnidii Rhizoma (3 g), and Rehmanniae Radix (3 g). Juzen-taiho-to has traditionally been used for patients with anorexia, anemia or fatigue, and is known to function as a biological response modifier.[6–10] We reported previously that Juzen-taiho-to reduced both the nephrotoxicity and bone marrow toxicity without reducing the antitumor effect of CDDP in mice, when administered perorally (*p.o.*) 30 min before CDDP at a dose of 10-fold the usual daily dose (1728 mg/kg).[11] This report describes the effects of the ingredients of Juzen-taiho-to on CDDP-induced nephrotoxicity and bone marrow toxicity in mice and the isolation of an active constituent, sodium L-malate, from Angelicae Radix, which exhibited the strongest inhibitory effect on the toxicity.

Results and Discussion

Effect of Ingredients of Juzen-taiho-to on CDDP-Induced Toxicity and Antitumor Effect of CDDP Table I summarizes the effects of ingredients of Juzen-taiho-to on CDDP-induced toxicity and the antitumor effect of CDDP against sarcoma 180 (S-180). The effects of the ingredients on the toxicity were examined on day 17, when the toxicity reached the maximum level in this animal model. An index of nephrotoxicity, blood urea nitrogen (BUN),[12–14] increased significantly to about 4 times the control level by treatment with CDDP. Of the 10 ingredients tested, 6 significantly reduced the increase in BUN at doses of 10 and 5 times the usual daily dose. Only Angelicae Radix exhibited a significant effect at the usual daily dose, and the effect was almost the same as that of Juzen-taiho-to itself. The ED_{50} of a water extract of Angelicae Radix was 17.8 mg/kg/d. None of the ingredients alone had any significant effect on BUN level (data not shown).

A decrease in white blood cell (WBC) count, which is an index of the bone marrow toxicity,[15–17] to 30% of the control value by the administration of CDDP was inhibited significantly by 4 of the 10 ingredients tested at a dose of 10 times the usual daily dose, and Angelicae Radix, Ginseng Radix, and Glycyrrhizae Radix showed a significant protective effect at a dose of 5 times the usual daily dose. None of the ingredients alone exhibited any significant effect on WBC count (data not shown).

CDDP alone showed 80.8% inhibition of the growth of S-180 cells. The ingredients did not exert any significant effect on the antitumor effect of CDDP except for the highest dose of the diuretic Hoelen,[18] which is known to reduce the antitumor effect of CDDP as well as its toxicity.[19,20] Each of the ingredients alone had no apparent antitumor effect on S-180 (data not shown).

CDDP alone showed a 34.5% loss in final body weight of mice compared with the control value. Treatment with the ingredients significantly reduced the weight loss induced by CDDP, except for Rehmanniae Radix. None of the ingredients alone had any significant effect on the body weight.

Isolation and Activity of Sodium L-Malate Figure 1 shows the procedure for isolating the constituent having protective effects against CDDP-induced nephrotoxicity.

TABLE I. Effect of Ingredients of Juzen-taiho-to on CDDP-Induced Toxicity and Antitumor Activity of CDDP

Treatment	Dose	Body weight loss		Nephrotoxicity		Bone marrow toxicity		Antitumor effect	
		Body weight (g)[a]	Inhibition (%)	BUN (mg/dl)[a]	Inhibition (%)	WBC ($\times 10^2$/mm^3)[a]	Inhibition (%)	Tumor weight (g)[a]	Inhibition (%)
Control	—	34.5±1.1	—	25.3± 1.9	—	60±5	—	1.15±0.11	—
CDDP alone	—	22.6±1.8###	—	99.6±14.1###	—	18±4###	—	0.22±0.05###	80.9
CDDP+Angelicae	1[b]	29.4±2.9***	57.1	60.2± 5.9*	53.0	22±5	9.5	0.23±0.05[e]	80.0
Radix	5[c]	31.8±3.3***	77.3	40.5± 5.5**	79.5	45±4**	64.3	0.23±0.05	80.0
	10[d]	33.2±2.5***	89.1	29.7± 3.6***	94.1	58±6***	95.2	0.24±0.05	79.1
CDDP+Ginseng	1	27.0±3.2***	37.0	66.2± 6.9	45.0	19±5	2.4	0.23±0.06	80.0
Radix	5	30.2±3.5***	63.9	43.2± 5.2**	75.9	39±4*	50.0	0.27±0.05	76.5
	10	33.0±2.5***	87.4	33.8± 4.9***	88.6	50±5***	76.2	0.28±0.05	75.7
CDDP+Hoelen	1	26.8±3.1**	35.3	66.2± 6.9	45.0	20±5	4.8	0.21±0.04	81.7
	5	28.6±4.2***	50.4	46.2± 5.1*	71.9	31±4	31.0	0.30±0.05	73.9
	10	32.2±2.9***	80.7	29.5± 2.1***	94.3	48±5**	71.4	0.40±0.09	65.2
CDDP+Glycyrrhizae	1	25.6±4.2*	25.2	70.9± 6.6	38.6	23±4	11.9	0.21±0.04	81.7
Radix	5	28.6±4.1***	50.4	50.2± 5.9*	66.5	41±6*	54.8	0.24±0.05	79.1
	10	31.2±2.5***	72.2	40.2± 4.0**	79.9	47±5**	69.0	0.23±0.06	80.0
CDDP+Astragali	1	24.8±2.8*	18.5	79.1± 7.2	27.6	18±4	0.0	0.23±0.06	80.0
Radix	5	27.6±3.0**	42.0	51.2± 4.9*	65.1	29±6	26.2	0.24±0.06	79.1
	10	30.5±4.2***	66.4	38.9± 5.1***	81.7	35±5	40.5	0.26±0.05	77.4
CDDP+Cinnamomi	1	24.1±4.6	12.6	77.0± 8.1	30.4	17±6	−2.4	0.22±0.04	80.9
Cortex	5	26.2±2.2*	30.5	52.9± 6.9*	62.9	26±5	19.0	0.29±0.05	74.8
	10	28.9±3.0***	52.9	42.2± 3.9***	77.3	31±4	31.0	0.30±0.07	73.9
CDDP+Atractylodis	1	23.9±2.6	10.9	88.2± 8.1	15.3	17±6	−2.4	0.27±0.04	76.5
Rhizoma	5	25.2±5.6*	21.8	70.2± 8.5	39.6	22±4	9.5	0.26±0.04	77.4
	10	27.2±4.2**	38.7	60.9± 8.1	52.1	31±5	31.0	0.29±0.06	74.8
CDDP+Paeoniae	1	23.1±2.6	4.2	90.2±10.1	12.7	20±4	4.8	0.26±0.04	77.4
Radix	5	24.2±4.2	13.4	81.3± 6.9	24.6	29±6	26.2	0.24±0.04	79.1
	10	26.2±5.2**	30.3	75.6± 5.9	32.3	36±6	42.9	0.24±0.04	79.1
CDDP+Cnidii	1	21.9±2.9	−5.9	90.9±10.6	11.7	22±5	9.5	0.24±0.05	79.1
Rhizoma	5	23.2±3.3	5.0	77.9± 7.6	29.2	31±6	31.0	0.27±0.06	76.5
	10	25.2±2.9*	21.8	71.5± 5.9	37.8	34±5	38.1	0.34±0.07	70.4
CDDP+Rehmanniae	1	21.9±2.9	−5.6	95.6±12.9	5.4	18±6	0.0	0.22±0.05	80.9
Radix	5	22.1±2.6	−4.2	92.9± 9.3	9.0	22±5	9.5	0.22±0.06	80.9
	10	23.2±3.2	5.0	92.6± 9.9	9.4	30±6	28.6	0.23±0.06	80.0
CDDP+Juzen-taiho-to	1	29.8±2.1***	58.0	58.6± 7.9*	55.2	31±7	31.0	0.23±0.06	80.0
	5	32.2±3.1***	80.7	42.9± 3.3**	76.3	48±5**	71.4	0.23±0.06	80.0
	10	33.2±2.5***	89.1	30.6± 2.9***	92.9	59±6***	97.6	0.22±0.05	80.9

All samples tested were administered *p.o.* to mice at doses of 1, 5, and 10 times the usual daily dose 30 min before CDDP i.p. injection. The control group was treated with water (*p.o.*) and saline (i.p.). S-180 cells were inoculated s.c. on day 1 and antitumor effect was determined on day 17. *a*) Mean±S.E. ($n=10$). *b*) The usual daily dose. *c*) Five times the usual daily dose. *d*) Ten times the usual daily dose. *e*) There was no significant difference between the CDDP alone group and the ingredient-treated groups. Significant difference from control group, ###: $p<0.001$. Significant difference from CDDP alone group, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

The purification was guided by BUN measurements. Table II summarizes the effects of fractions from Angelicae Radix on CDDP-induced nephrotoxicity in mice.

As the first step of the isolation procedure, two crude residues were obtained by partition with BuOH–H_2O. The inhibitory effect was detected mainly in the BuOH layer. Only the H_2O eluate from the BuOH layer proved to be active, so it was submitted to further purification by ultrafiltration to obtain a more active fraction with low molecular weight (LMF). Among the fractions separated from LMF, only fr. 40 showed a significant inhibitory effect on the toxicity. The fr. 40 was further subjected to column chromatography on Sephadex G-15. The inhibitory effect was detected in frs. G 59, 60, 61 and 62. Finally, the most active fractions, frs. G 60 and 61, were purified by Sephadex G-10 column chromatography. Sodium L-malate, $C_4H_4Na_2O_5$, was obtained as an amorphous powder from fr. S 61, which exhibited the strongest inhibitory effect on CDDP-induced toxicity.

Table III summarizes the effects of sodium L-malate isolated from Angelicae Radix on the CDDP-induced toxicity. Sodium L-malate dose-dependently prevented the increase in BUN induced by CDDP and the ED_{50} was 0.4 mg/kg. Sodium L-malate also dose-dependently prevented the decrease in the WBC count and the ED_{50} was 1.8 mg/kg. CDDP alone caused 79.3% inhibition of the growth of S-180 cells. Sodium L-malate did not show any significant effect on the antitumor activity of CDDP.

CDDP alone caused a 32.8% loss in body weight of mice compared with the control value. Treatment with over 0.89 mg/kg sodium L-malate significantly inhibited the weight loss by CDDP. Sodium L-malate alone did not exert any significant effect on the BUN, WBC, antitumor effect or body weight (data not shown).

The ED_{50} of sodium L-malate for protection against the nephrotoxicity was 44.5 times lower than that of Angelicae Radix. The ED_{50} of sodium L-malate for protection against the bone marrow toxicity was also 33 times lower than that of Angelicae Radix. In addition, the ratio of the ED_{50} for the nephrotoxicity to that for the

bone marrow toxicity in sodium L-malate was similar to that in Angelicae Radix. Commercial sodium L-malate showed the same inhibitory effect as that of the isolated sodium L-malate (data not shown). These findings indicate that sodium L-malate is an important constituent compound in the inhibitory effect of Angelicae Radix against the nephrotoxicity and bone marrow toxicity of CDDP.

Further studies to elucidate the role of sodium L-malate in the action of Angelicae Radix and Juzen-taiho-to are in progress.

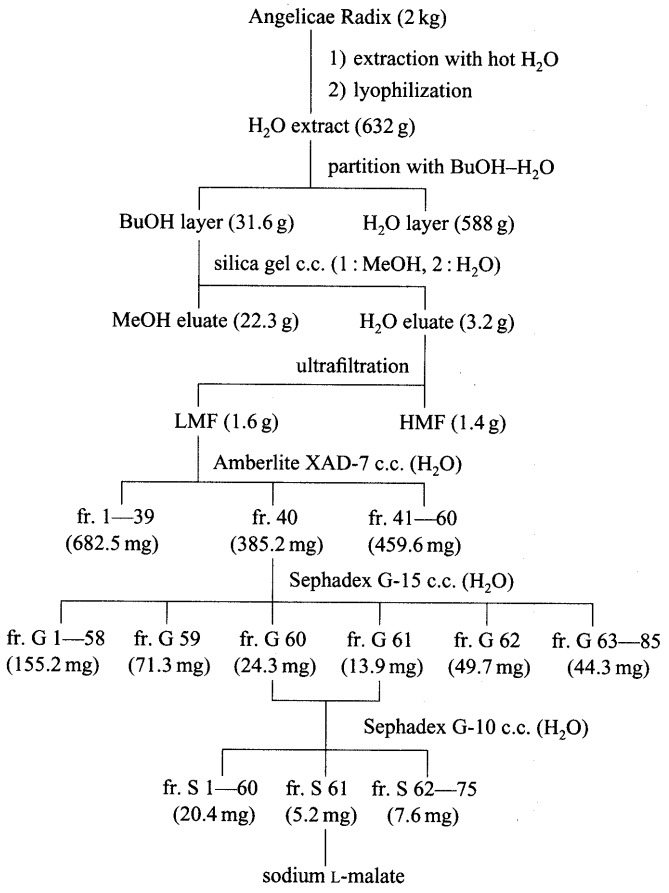

Fig. 1. Isolation Procedure for Sodium L-Malate from Angelicae Radix

Isolation was performed, guided by BUN measurements in mice. The yield of each fraction obtained from 2 kg of Angelicae Radix is designated in parentheses. LMF and HMF indicate low-molecular-weight fraction (MW < 10000) and high-molecular-weight fraction (MW > 10000), respectively.

Conclusion

The present examination indicates that Angelicae Radix and its constituent, sodium L-malate, provide significant protection against CDDP-induced nephrotoxicity and bone marrow toxicity without reducing the antitumor activity. Further studies to clarify the mechanism of protection against CDDP-induced toxicity by sodium L-malate are being conducted in our laboratory.

Experimental

Measurements The IR spectra were taken with a JASCO A-202 spectrophotometer, and the FAB-MS were recorded on a JEOL JMS-SX102 spectrometer. The ^1H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 270 spectrometer (270 MHz), and chemical shifts are given in δ with 3-(trimethylsilyl)propanesulfonic acid sodium salt as an internal standard. Optical rotations were measured with a JASCO DIP-140 polarimeter. The concentration of sodium was detected by use of an inductively coupled plasma spectrometer (ICP) (Seiko SPS1200A). The WBC count was made on a Celltac 4150 (Nihon Koden, Ltd., Tokyo, Japan), and BUN was measured spectrometrically on a COBAS FARA (Baxter, Ltd., Tokyo) using an assay kit for urea nitrogen-HR (Wako Pure Chemical Industries, Ltd., Tokyo).

Chemicals CDDP was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sodium L-malate, $C_4H_4Na_2O_5 \cdot xH_2O$, was obtained from Wako Pure Chemical Industries, Ltd.

Animals Five-week-old, male, ddY mice (average weight, 25 g) were obtained from Japan SLC, Inc., Hamamatsu, Japan, and kept in rooms with a controlled temperature (23 ± 0.5 °C), humidity (50 ± 5%), and 12-h

TABLE II. Effect of Various Fractions of Angelicae Radix on the Nephrotoxicity of CDDP

Fractions	Dose (mg/kg)	BUN (mg/dl)[a]	Inhibition (%)
Control	—	26.8 ± 0.9	
CDDP alone	—	94.5 ± 9.5###	
CDDP + H_2O extract	200	31.5 ± 2.5***	93.1
CDDP + BuOH layer	20	29.5 ± 1.7***	96.0
CDDP + H_2O eluate	2	30.8 ± 1.9***	94.1
CDDP + LMF	1	32.8 ± 2.9***	91.1
CDDP + fr. 40	0.5	34.5 ± 3.1***	90.1
CDDP + fr.G 60	0.25	42.2 ± 5.7**	77.3
CDDP + fr.G 61	0.25	46.2 ± 6.9*	71.3
CDDP + fr.S 61	0.20	48.9 ± 6.5*	67.4

All the fractions tested were given *p.o.* to mice 30 min before CDDP (0.01 mmol/kg) i.p. injection. The control group was treated with water (*p.o.*) and saline (i.p.). *a*) Mean ± S.E. ($n = 10$). Significant difference from the control group, ###: $p < 0.001$. Significant difference from the CDDP alone group, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

TABLE III. Effect of Sodium L-Malate on CDDP-Induced Toxicity and Antitumor Activity of CDDP

Treatment	Dose (mg/kg)	Body weight loss: Body wt.[a] (g)	Body weight loss: Inhibition (%)	Nephrotoxicity: BUN[a] (mg/dl)	Nephrotoxicity: Inhibition (%)	Bone marrow toxicity: WBC[a] ($\times 10^2/mm^3$)	Bone marrow toxicity: Inhibition (%)	Antitumor activity: Tumor wt.[a] (g)	Antitumor activity: Inhibition (%)
Control	—	33.5 ± 1.9		27.5 ± 0.9		62 ± 8		1.16 ± 0.30	
CDDP alone	—	22.5 ± 2.9###		95.9 ± 9.9###		23 ± 5###		0.24 ± 0.15###	79.3
CDDP + sodium L-malate	0.22	22.4 ± 2.8	−0.9	53.7 ± 9.2	61.7	31 ± 11	20.5	0.28 ± 0.16[b]	75.9
	0.45	23.9 ± 2.1	12.7	48.9 ± 7.2**	68.7	35 ± 12	30.8	0.29 ± 0.17	75.0
	0.89	26.8 ± 2.6*	39.1	37.5 ± 3.4***	85.4	40 ± 9	43.6	0.30 ± 0.13	74.1
	1.78	29.4 ± 2.8***	62.7	35.5 ± 3.5***	88.3	42 ± 10*	48.7	0.30 ± 0.16	74.1
	3.56	31.8 ± 2.9***	84.5	32.3 ± 3.0***	93.0	45 ± 12*	56.4	0.32 ± 0.20	72.4

Sodium L-malate (0.00125—0.02 mmol/kg) was given *p.o.* to mice 30 min before CDDP (0.01 mmol/kg) i.p. injection. The control group was treated with water (*p.o.*) and saline (i.p.), and antitumor activity was determined on day 17. *a*) Mean ± S.E. ($n = 10$). *b*) There was no significant difference between the CDDP alone group and sodium L-malate-treated groups. Significant difference from the control group, ###: $p < 0.001$. Significant difference from the CDDP alone group, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

light/12-h dark cycles.

Treatment of Animals Each test group comprised 10 mice. The animals were inoculated with S-180 cells (10^6/mouse) in the left thigh subcutaneously on day 1. CDDP (3 mg/kg) was given i.p. to the mice on days 3, 4, 5, 6, 7, 8, 10, 11 and 12. Test samples were given *p.o.* to the mice on days 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 and 15. The control group was treated with water (*p.o.*) and saline (i.p.). On day 17, mice were anesthetized with ether, then blood was collected from the inferior vena cave using a heparinized syringe, and the number of WBC was immediately counted. After centrifugation of the remaining blood, the serum was analyzed for BUN, and the tumor was resected and weighed. Student's *t* test was used to evaluate the significance of differences between experimental groups.

The inhibitory rate of test samples on the nephrotoxicity of CDDP was calculated by use of the following formula: Inhibitory rate $(\%)=\{1-(B-C)/(A-C)\}\times 100$, where A, B and C are the mean values of BUN of CDDP alone, test samples and the control, respectively. The inhibitory rate of the test samples on the bone marrow toxicity and the body weight loss was calculated by using the following formula: Inhibitory rate $(\%)=(B-C)/(A-C)\times 100$, where A, B and C are the mean values of WBC count or body weight of the control, test sample and CDDP alone groups, respectively. The inhibitory rate of the antitumor effect was also calculated by using the following formula: Inhibitory rate $(\%)=(1-B/A)\times 100$, where A is the mean tumor weight of the control and B is that of CDDP alone or test sample groups.

Preparation of the Water Extract of Ingredients The ingredients of Juzen-taiho-to were purchased from Tsumura & Co., Tokyo. A usual daily dose of Angelicae Radix (3 g), Hoelen (3 g), Glycyrrhizae Radix (2 g), Ginseng Radix (3 g), Astragali Radix (3 g), Cinnamomi Cortex (3 g), Atractylodis Rhizoma (3 g), Paeoniae Radix (3 g), Cnidii Rhizoma (3 g) or Rehmanniae Radix (3 g) was extracted with boiling water for 60 min. After cooling, the extract was filtered, and then lyophilized. The lyophilized material was dissolved in water immediately before use. The yield of each water extract obtained from the usual daily dose of the ingredient was as follows: Angelicae Radix (0.99 g, 33.0%), Hoelen (0.04 g, 1.2%), Glycyrrhizae Radix (0.52 g, 26.2%), Ginseng Radix (1.0 g, 36.8%), Astragali Radix (0.77 g, 25.6%), Cinnamomi Cortex (0.17 g, 5.5%), Atractylodis Rhizoma (1.05 g, 35.1%), Paeoniae Radix (0.56 g, 18.6%), Cnidii Rhizoma (0.74 g, 24.9%), and Rehmanniae Radix (1.45 g, 48.3%). In animal experiments, the usual daily dose of the water extract was calculated on the basis of the yield of each ingredient and an average human body weight of 60 kg.

Isolation of Sodium L-Malate The dried roots of Angelicae Radix (2 kg), cultivated in Gunma prefecture, Japan, were extracted with 20 l of water under boiling for 60 min. After cooling, the extract was filtered, and then lyophilized to give the H_2O extract (632 g, 31.6%). The extract (63.2 g) was then partitioned into a BuOH–H_2O (1 : 1) mixture (2 l). Removal of the solvent from the H_2O layer and the BuOH layer under reduced pressure below 40 °C yielded the H_2O layer (58.8 g, 93.0%) and the BuOH layer (3.16 g, 5.0%). In total, 31.6 g of the BuOH layer was obtained by repeating the partition. The BuOH layer (16.0 g) was subjected to silica gel column chromatography (800 g) with MeOH (7 l) and H_2O (7 l) as eluents, giving two fractions, the MeOH eluate (11.7 g, 73.1%) and the H_2O eluate (1.6 g, 10.0%). In total, 3.2 g of the H_2O eluate was obtained by repeating the chromatography. Ultrafiltration (Diaflo Ultrafiltration Membranes YM-10, 150 mm diameter, Amicon Co.) of the H_2O eluate (1.0 g in 1000 ml of H_2O) afforded a low-molecular-weight fraction LMF (MW < 10000, 0.49 g, 49.0%) and a high-molecular-weight fraction (HMF, MW > 10000, 0.44 g, 44.0%). In total, 1.6 g of the LMF was obtained by repeating the ultrafiltration. The LMF (1.6 g) was subjected to column chromatography on Amberlite XAD-7 (450 g, Orugano Co.) with water as an eluent to give 60 fractions (20 ml each). The active fraction fr. 40 was obtained in a yield of 385.2 mg (24.1%). The fr. 40 (100 mg) was further purified by Sephadex G-15 column chromatography (2.4 × 130 cm, Pharmacia Fine Chemicals) with water as an eluent to give 85 fractions (4 ml each). The active fractions frs. G 60 and 61 were obtained in yields of 6.3 mg (6.3%) and 3.6 mg (3.6%), respectively. In total, 38.2 mg of frs. G 60 and 61 was obtained by repeating the chromatography. The frs. G 60 and 61 (20 mg) were subjected again to Sephadex G-10 column chromatography (2.4 × 130 cm) with water as an eluent to give 75 fractions (4 ml each), and an active constituent sodium L-malate was obtained in a yield of 2.7 mg (13.5%) from fr. S-61 as an amorphous powder. In total, 5.2 mg of sodium L-malate was obtained by repeating the chromatography.

Sodium L-Malate $[\alpha]_D$ +8.2 (c = 2.0, H_2O, 25 °C). FAB-MS m/z: 179 $(M+H)^+$. IR γ_{max}^{KBr} cm^{-1}: 3400 (–OH), 1685 (C=O). ^{1}H-NMR (D_2O) δ: 2.38 (1H, dd, J = 15.6, 10.4 Hz, –C<u>H</u>$_2$–CH), 2.69 (H, dd, J = 15.6, 3.1 Hz, –C<u>H</u>$_2$–CH), 4.32 (1H, dd, J = 10.4 Hz, 3.1 Hz, –CH$_2$–C<u>H</u>). ^{13}C-NMR (D_2O) δ: 43.6 (t, –CH_2–), 71.4 (d, –CH–), 180.9, 182.1 (s, C=O). ICP: Na; 25.4%.

Sodium L-malate was identified by comparison of optical rotation, IR, ^{1}H-NMR, and ^{13}C-NMR data with those of an authentic sample obtained from Wako Pure Chemical Industries, Ltd.

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