

Augmentation of the Pharmacological Action of *Corydalis* Tuber by *Saussurea* Root in Isolated Mouse Ileum

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To understand the meaning of blending crude drugs in Chinese medicinal prescriptions, the influence of *Saussurea* root on the pharmacological action of *Corydalis* tuber was examined. *Saussurea* root increased the depression of acetylcholine-induced contraction caused by the hot water extract solution of *Corydalis* tuber in mouse ileum at low dosage, which showed no direct influence on acetylcholine. Dehydrocostuslactone in *Saussurea* root was characterized as the component having increasing activity and the relationship between the concentration of acetylcholine and the variation in the contraction depressed by *Corydalis* tuber alone or a mixture of the *Corydalis* tuber and dehydrocostuslactone was investigated for clarification of the mode of action.

Key words—*Saussurea* root; *Corydalis* tuber; dehydrocostuslactone; acetylcholine; ileum; mouse

Corydalis tuber, contains alkaloids^{1–4)} as its principal constituents, is blended in several Chinese medicinal prescriptions. This crude drug is considered chiefly as an analgesic against abdominal pains in oriental medicine and is also blended in general gastroenteric OTC drugs as an analgesic and antispasmodic. The alkaloids of *Corydalis* tuber have previously been reported as antispasmodic active ingredients.⁵⁾ A hot water extract solution of this crude drug depressed contraction of the isolated ileum of mice induced by acetylcholine (ACh) in an organ bath. As *Saussurea* root is blended together with *Corydalis* tuber in some Chinese medicinal prescriptions such as Goshitsu-san, Kisyuku-nichin-to and Shimko-to, the possibility of potentiation in pharmacological action exists. In this work, we found that *Saussurea* root enhanced this depressive action of *Corydalis* tuber at low dosages, which singly showed no influence on the ileum. In addition, we attempted characterization of the constituents in *Saussurea* root, which increase the depressive action of *Corydalis* tuber on ACh-induced contraction.

SUBJECTS AND METHODS

Materials Chopped *Corydalis* tubers (from China) and *Saussurea* roots (from China) were purchased from Nakai-kohshindo (Kobe, Japan). Both crude drugs used were those defined by **JP XIII**. Acetylcholine chloride and reagents for Tyrode's solution were purchased from Nacalai tesque (Kyoto).

Animals Six- to 7- week-old male mice of the ddY strain were purchased from Nihon SLC, Hamamatsu, Japan. They were housed in plastic cages and kept in a room at $25 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity with a 12h dark-light cycle. The animals were starved for one day before use but allowed free access to water until the beginning of the experiment.

Action on isolated mouse ileum According to the Magnus method, an ileum preparation isolated from a mouse was suspended in a 50ml organ bath filled with Tyrode's solution (temperature, $26\text{--}28^\circ\text{C}$). A concentration of 0.1 ml of ACh dissolved in Tyrode's solution was applied to the bath. The contraction of the ileum was amplified by a transducer (Natsume KN-259) and recorded on a Kimorecorder. The materials to be examined were applied to the bath 3 min before ACh administration and contractions were compared.

Test samples Six g of *Corydalis* tuber or *Saussurea* root was refluxed with 60 ml of water (10 times) for 30 min and filtered through absorbent cotton. The extract was concentrated to 10 ml *in vacuo* and 0.5 ml was applied to the organ bath before administration of ACh (contents of extracts: *Corydalis* tuber, 1.04 g/10 ml; *Saussurea* root, 2.97 g/10 ml). The mixture of *Corydalis* tuber and *Saussurea* root (each 6 g) was similarly refluxed with 120 ml of water for 30 min and concentrated to 20 ml, then 1.0 ml of the solution was applied to the bath (content of extract, 4.07 g/20 ml). Methanolic extract or isolated

components of *Saussurea* root were dissolved or suspended (using Tween 80) in 5 ml of water at the concentration showing no direct influence on ACh action, then mixed with 5 ml of hot water extract solution of *Corydalis* tuber alone, mentioned above. Then 1.0 ml of each mixture solution was applied to the organ bath. Each chromatographed fraction (dosages were calculated based on the yields from the methanolic extract) was also dissolved or suspended (using Tween 80) in 5 ml of water and investigated in a similar fashion.

Extraction and fractionation *Saussurea* roots (100 g) were refluxed with methanol and filtered through absorbent cotton. Removal of solvent *in vacuo* gave methanolic extract (16.1 g). The extract was dissolved in water and successively distributed between chloroform, ethyl acetate and *n*-butanol. After removal of the respective solvents, the individual extracts were obtained. The chloroform extract (4.0 g), indicating increased activity, was column chromatographed on silica gel (Wakogel C-200) to give two colorless substances. They were identified as dehydrocostuslactone (1) (160 mg) and costunolide (2) (98 mg) by comparison of the infrared absorption spectra and behaviors on thin-layer chro-

matography and high-performance liquid chromatography with those of authentic compounds, which we have previously isolated and reported.⁶

Statistics Statistical analysis was performed by Student's *t*-test. Values of $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

The addition of a hot water extract solution of *Corydalis* tuber had an approximately 45%, on average, relaxing action on the ACh (1×10^{-7} g/ml)-induced contraction of the mouse ileum. Since *Corydalis* tuber and *Saussurea* root are blended and decocted together in some Chinese medicinal prescriptions, application of a hot water extract solution of these mixed two crude drugs (1 : 1) was examined, and it was found that this depression of ACh-induced contraction was further augmented up to about 65%. On the other hand, a hot water extract solution of *Saussurea* root alone showed little influence on the ileum and ACh-induced contraction (Fig. 1).

Methanolic extract of *Saussurea* root also showed this increasing effect on the action of *Corydalis* tuber below dosages having little influence on the

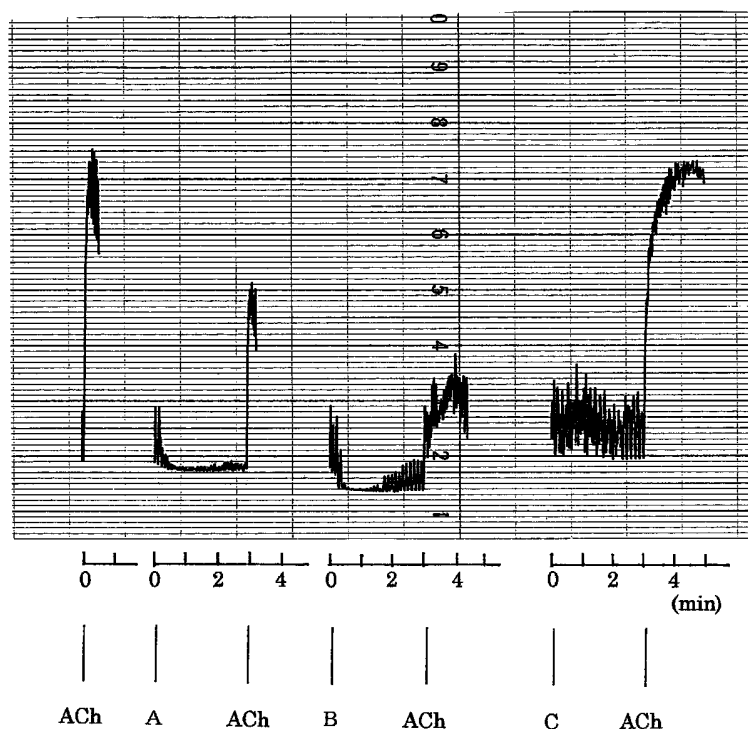


Fig. 1. Change in Contractions of Mouse Ileum Caused by 1×10^{-7} g/ml of Acetylcholine

A, hot water extract solution of *Corydalis* tuber alone; B, hot water extract solution of mixed *Corydalis* tuber and *Saussurea* root (1 : 1); C, hot water extract solution of *Saussurea* root alone.

ACh activity. The augmentation of the depression by aqueous solution of the methanol extract of *Saussurea* root (30 mg/5 ml) in a case mixed with a hot water extract solution of *Corydalis* tuber (1 : 1) is shown in Fig. 2.

The aqueous solution of the methanolic extract was subsequently partitioned between several organic solvents and results indicated that the chloroform extract showed the increasing effect. This extract was further fractionated by column chromatography, and the isolated compound **1** was the active ingredient.

Sesquiterpenoids⁷⁻¹⁰⁾ are known as representative constituents of *Saussurea* root. It was previously reported that **1**, one of these principal sesquiterpenes, exhibited a depression of carbachol-induced contraction in the colon of rats *in vitro*.¹¹⁾ However, 2 mg/5 ml of an aqueous suspension of **1** showed no influence on ACh action in an organ bath because of its lower concentration. As shown in Fig. 3, the mixture of this suspension and the hot water extract solution of *Corydalis* tuber (1 : 1) increased the depression of ACh-induced contraction of the latter alone by about 55% on average. Compound **2**, another principal

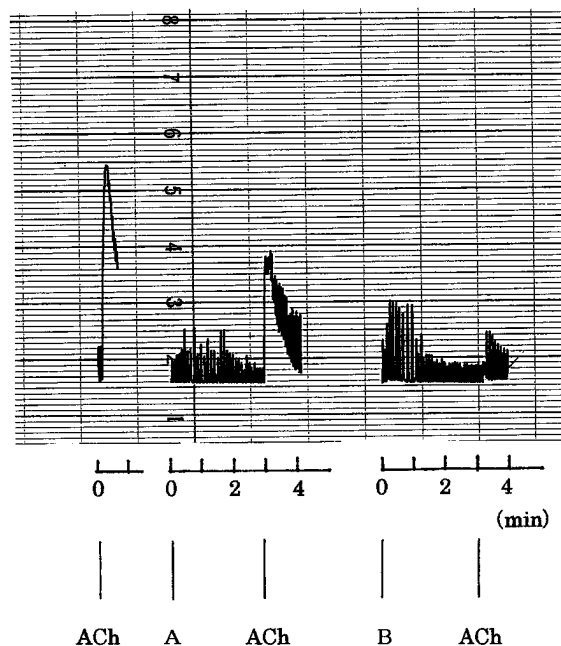


Fig. 2. Contraction of Mouse Ileum after Addition of Extracts

ACh, 1×10^{-7} g/ml; A, hot water extract solution of *Corydalis* tuber alone; B, mixture of hot water extract solution of *Corydalis* tuber and aqueous solution of methanolic extract of *Saussurea* root (30 mg/5 ml, 1 : 1).

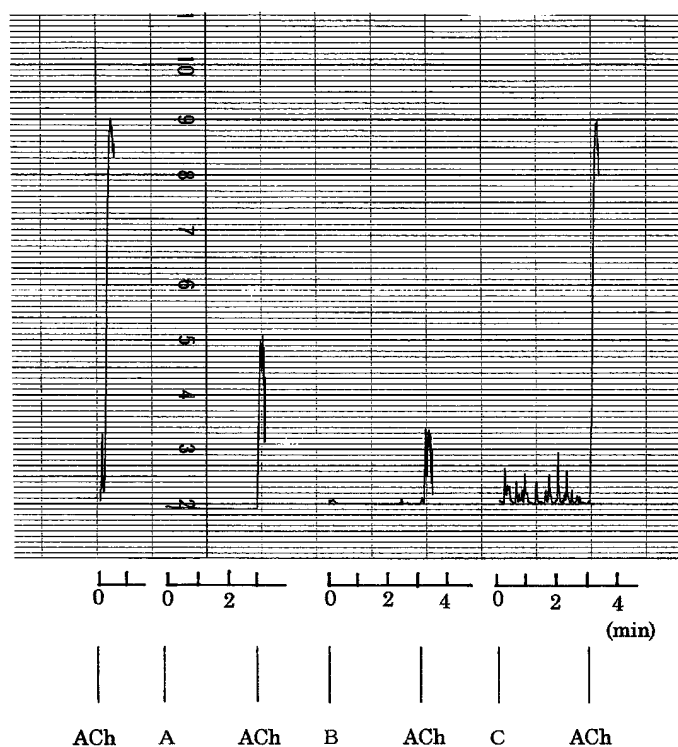


Fig. 3. Comparison of the Effects of Mixtures of *Corydalis* Tuber and Dehydrocostuslactone with that of *Corydalis* Tuber Alone on the Contraction of Mouse Ileum Induced by 1×10^{-7} g/ml of Acetylcholine

A, hot water extract solution of *Corydalis* tuber alone; B, mixture of a hot water extract solution and aqueous suspension of 2 mg/5 ml of dehydrocostuslactone (1 : 1); C, aqueous suspension of 2 mg/5 ml of dehydrocostuslactone alone.

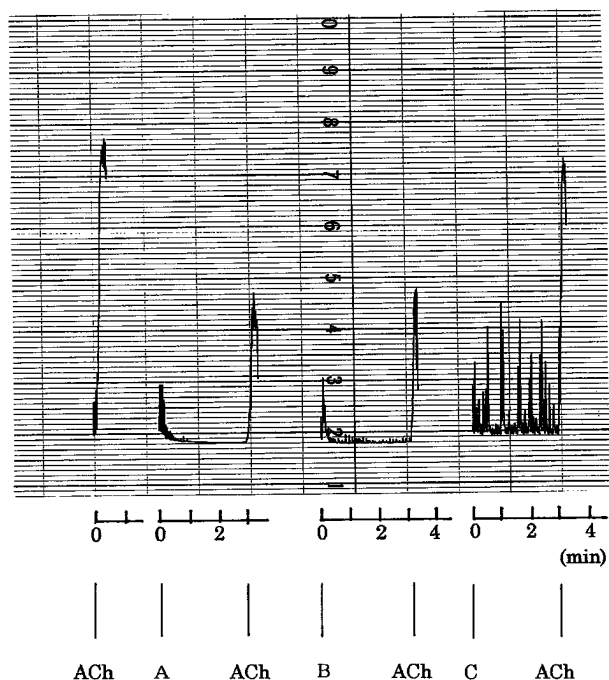


Fig. 4. Comparison of the Effects of Mixtures of Corydalis Tuber and Costunolide with that of Corydalis Tuber Alone on the Contraction of Mouse Ileum Induced by 1×10^{-7} g/ml of Acetylcholine

A, hot water extract solution of Corydalis tuber alone; B, mixture of hot water extract solution and aqueous suspension of 5 mg/5 ml of costunolide (1 : 1); C, aqueous suspension of 5 mg/5 ml of costunolide alone.

constituent, also had a depressive activity on carbachol-induced contraction.¹¹⁾ However, its lower concentration, 5 mg/5 ml of aqueous suspension, exhibited no influence on ACh action and scarcely increased the Corydalis tuber's action (Fig. 4).

Changes in the contraction rate of mouse ileum when the concentration of ACh was altered were compared with addition of hot water extract solution of Corydalis tuber alone or the mixed solution with 1 (2 mg/5 ml, 1 : 1). Findings are shown in Fig. 5. Compared with ACh alone, each contraction did not shift in parallel. Therefore, this antagonism is implied to be papaverine-like non-competitive antagonism.

Similar to Corydalis tuber, Saussurea root is blended in gastroenteric drugs on the market as an aromatic stomachic. The findings mentioned above suggest that coexistent Saussurea root enhances the action of Corydalis tuber even when dispensed in a decoction together. It was also suggested that Saussurea root itself works with an antispasmodic action along with Corydalis tuber at high concentration and supports the action of Corydalis tuber even at low concentration.

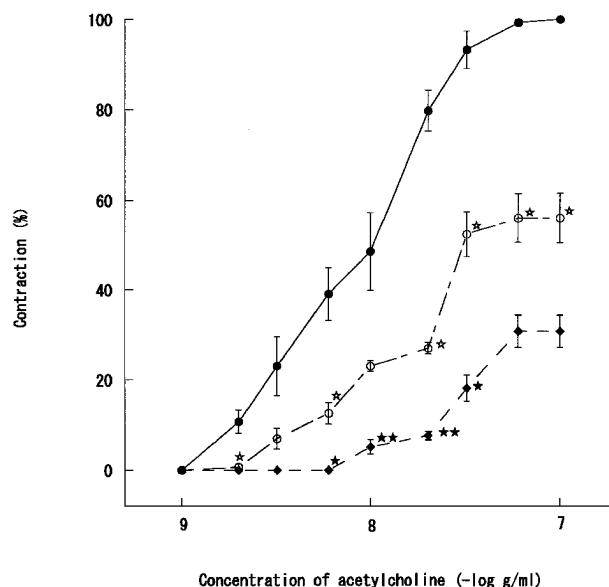


Fig. 5. Effects of Corydalis Tuber and Mixtures of Corydalis Tuber and Dehydrocostuslactone against Contractions of Mouse Ileum Induced by Various Concentrations of Acetylcholine.

Each point represents the mean \pm S.E. ($n=3$). Significantly different versus each value at each concentration, \star : $p < 0.05$ (from \bullet); $\star\star$: $p < 0.01$ (from \circ). \bullet , ACh alone; \circ , ACh and addition of a hot water extract solution of Corydalis tuber; \blacklozenge , ACh and addition of a mixture of a hot water extract solution of Corydalis tuber and aqueous suspension of dehydrocostuslactone. The contraction induced by 1×10^{-7} g/ml of ACh alone was defined as 100% reaction.

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