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Studies on the Pharmaceutical Quality Evaluation of Crude Drug Preparations used in Orient Medicine "Kampoo". III.[1] Precipitation Reaction of Glycyrrhizin with Alkaloids or Alkaloidal Crude Drugs in Aqueous Solution

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Precipitation reaction of glycyrrhizin (I) with 32 kinds of alkaloids including synthetic bases and 10 kinds of crude drugs which contain various types of alkaloids in aqueous solution was investigated and following results were obtained. 1) Tertiary alkaloids which had heterocyclic aromatic rings or nitrogen atoms neighbouring to aromatic ring or conjugated olefine systems in the structure, namely, papaverine, noscapine, emetine, O-methyl domesticine, domesticine, nornuciferine, *dl*-tetrahydroberberine, *l*-corydaline, quinine, reserpine and sinoactine gave precipitate, while other types of alkaloids, namely, *l*-ephedrine, N-methylephedrine, *l*-phenylalaninol, atropine, *l*-scopolamine, cocaine, morphine, ethylmorphine, codeine, hydrocodeine, thebaine, sinomenine, aconitine, physostigmine, caffeine, pethidine did not. 2) Tertiary alkaloids which had pK_a values smaller than 7.5 were likely to precipitate with I, while those which had pK_a values larger than 7.5 did not. 3) Among quaternary alkaloids, berberine and coptisine produced precipitate, while menisperine, N-methyl scopolamine and neostigmine did not. 4) Among crude drugs which contained various alkaloids, coptis rhizome, corydalis roots, phellodendron cortex and evodia fruits gave precipitate with I in aqueous solution, while aconite roots, areca seeds, ephedra herb, magnolia cortex, nupharis rhizome and sinomenium roots did not.

Keywords——precipitation reaction of glycyrrhizin and alkaloids; glycyrrhizin; quality evaluation of crude drug preparations; alkaloidal crude drugs; ionization constant of alkaloids; decoction of crude drugs; HSLC

In the previous papers, it was revealed that berberine (I) in coptis rhizome and glycyrrhizin (II) in glycyrrhiza radix produced precipitates in aqueous solution in which two mol of I were ionically bonded on the glucuronic acid moieties of II.[1,3]

Many kinds of crude drugs which contain alkaloids are used in orient medicine "Kampoo", so it is important to investigate whether this type of precipitation reaction occurs between these drugs and glycyrrhiza radix in aqueous solution under boiling condition or not, for both the quantitation of such preparations and clarification of the pharmaceutical peculiality of them.

This paper deals with the precipitation reaction of II with 32 kinds of alkaloids and with 10 kinds of crude drugs commonly used in Kampoo which contain different types of alkaloids.

Experimental

The specific conductance was measured on Metrhom Konduktoskop E-365B with Electrode EA-608. Ultraviolet (UV) spectra were determined by Hitachi Double Wave Length Spectrophotometer Model 556. Infrared (IR) spectra were obtained with Hitachi Spectrophotometer Model G-2. Ionization constants

1) Part II: M. Noguchi, *Chem. Pharm. Bull.* (Tokyo), **26**, 2624 (1978).
2) Location; *a) 6, Hoenzaka-machi, Higashi-ku, Osaka, 540, Japan*; *b) 380, Nishiyama, Sayama-cho, Minamikawachi, Osaka, 589, Japan.*
3) M. Noguchi, M. Kubo, T. Hayashi, and M. Ono, *Shoyaku*, **32**, 104 (1978).

were determined with Beckman Zeromatic SS-3 pH Meter. High speed liquid chromatography (HSLC) determination was carried out by Waters Model 440 apparatus.

Measurement of Ionization Constant——10^{-4} mol of free alkaloids was dissolved in 10 ml MeOH and after addition of 10 ml of water, pH change was observed on each addition of 0.2 ml 0.01 N HCl. The pK_a values were graphycally obtained from the titration curves.[4]

Observation of IR Spectra——The precipitate made from alkaloid and glycyrrhizin (II) was centrifuged and washed with a small quantity of distilled water. Ethanolic solution of the precipitate was filtered and evaporated *in vacuo*. IR spectra of the residues were measured by KBr tablet method.

Measurement of the Specific Conductance——10^{-3} M solution of ephedrine and II were mixed with different ratio as shown in Fig. 3 and the specific conductance was measured.

Determination of Noscapine (III) by UV Absorption Method——Each 1.02 mg of the precipitate from II and III, 0.64 or 0.32 mg of III was dissolved in 5 ml of MeOH and UV absorption was measured at 312 nm.

Determination of Corydaline (V) and Tetrahydroberberine (IV) by HSLC——Each 1.4 mg of the precipitate of IV or V with II, 0.5 and 0.3 mg of IV or V were dissolved in 5 ml of MeOH and determined by HSLC method. Operating conditions are as follows. Column; Bondapak C_{18}, 2 mm × 60 cm. solvent; MeOH: 1% AcOH, 8: 2. flow rate; 8.1 ml/min. Detection; UV 280 nm. Injection volume; 5 μl.

Table I. Precipitation Reaction of Glycyrrhizin and Alkaloids in Aqueous Solution

Group	Sample	Precip. react.	pK_a value
A	Emetine	+	7.36, 8.23[c]
	Noscapine	+	5.82[a]
	Papaverine	+	5.75[a]
B	Domesticine	+	
	Nornuciferine	+	
	O-methyldomesticine	+	7.08[a]
C	*l*-Corydaline	+	6.45[a]
	dl-Tetrahydroberberine	+	
D	Quinine	+	4.30, 8.93[c]
	Reserpine	+	6.6[c]
E	*l*-Ephedrine	−	8.93,[a] 9.46[b]
	N-Methylephedrine	−	8.60[a]
	l-Phenylalaninol	−	8.33,[a] 8.86[b]
F	Atropine	−	9.65[c]
	Cocaine	−	8.41[c]
	l-Scopolamine	−	
G	Codeine	−	7.95[c]
	Ethylmorphine	−	
	Hydrocodeine	−	
	Morphine	−	7.87[c]
	Sinoactine	+	6.35[a]
	Sinomenine	−	7.75[a]
	Thebaine	−	7.95[c]
H	Aconitine	−	
	Caffeine	−	
	Pethidine	−	
	Physostigmine	−	6.12, 12.25[c]
I	Berberine	+	
	Coptisine	+	
	Menisperine	−	
	Methylscopolamine	−	
	Neostigmine	−	

a) 50% MeOH was used as the solvent.
b) Distilled water was used as the solvent.
c) The value was described in Merck Index (1977).

4) S. Matsuura (ed.), "Ionization Constants," Maruzen, Tokyo, 1963.

Results and Discussion

I. Precipitation Reaction of Alkaloids and Glycyrrhizin (II) in Aqueous Solution

By adding 2 ml of saturated solution of II (K salt) on the solution of 5 mg alkaloids in 2.5 ml water for their salts or in the mixture of 2 ml of 0.02 N HCl and 0.5 ml EtOH for free bases, precipitation was observed in some cases and not in other cases. As shown in Table I, tertiary alkaloids which have heterocyclic aromatic rings or nitrogen atoms neighbouring to aromatic ring or conjugated olefine systems in the structure, namely, alkaloids of benzyl isoquinoline type (A), aporphine type (B), tetrahydroberberine type (C), quinine and reserpine (D) produced precipitates, while no precipitate was produced from the compounds which have nitrogen atom apart from aromatic ring or conjugated olefine systems in the structure, namely alkaloids of phenylalkylamine type (E), tropane type (F), morphine type (G), aconitine and petidine (H). Among quaternary alkaloids in group (I), berberine and coptisine produced precipitate, while menisperine, methyl scopolamine and neostigmine did not.

The pK_a values of these alkaloids were observed titrimetrically with 0.01 N HCl in aqueous or in 50% MeOH solution. Titration curves and pK_a values obtained from these curves[4] are shown in Fig. 1 and Table I, respectively.

The factors which influences the pK_a values of simple amines or alkaloids are believed to be operating in the case: (a) the electron-donating inductive effect of alkyl or other substituent groups, (b) steric shielding of solvation, (c) steric inhibition or resonance effect and (d) intramolecular hydrogen bonding.[5]

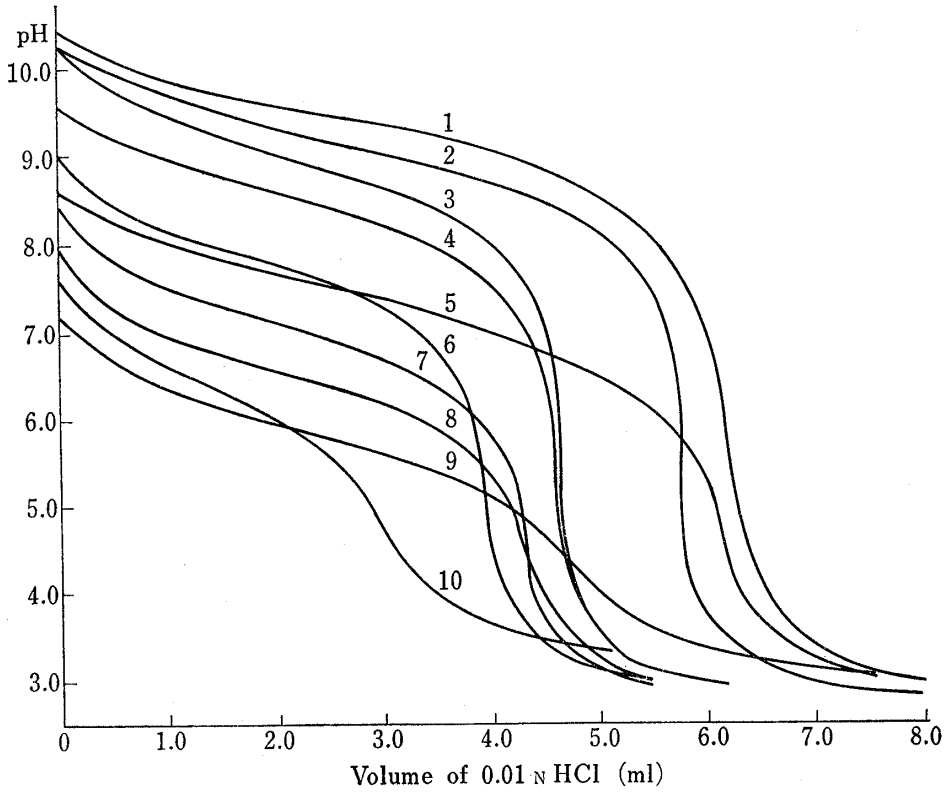

Fig. 1. Titration Curves of Various Alkaloids with 0.01 N Hydrochloric Acid

1: ephedrine (H_2O),
2: N-methylephedrine (50% MeOH),
3: phenylalaninol (H_2O),
4: phenylalaninol (50% MeOH),
5: scopolamine (50% MeOH),
6: sinomenine (50% MeOH),
7: O-methyldomesticine (50% MeOH),
8: sinoactine (50% MeOH),
9: noscapine (50% MeOH),
10: corydaline (50% MeOH).

5) A. Weissberger, "Technique of Organic Chemistry," Vol. XI. Elucidation of Structures by Physical and Chemical Methods, Intersciences, New York, 1963, p. 335, 367.

As shown in Table I, lowering effect of pK_a values by addition of MeOH in the solvent is observed in the case of ephedrine and phenylaninol. Steric or intramolecular hydrogen bonding effect is suggested to operate in the case of sinoactine that has much lower pK_a value than other compounds of morphine type.

The results shown in Table I indicate that alkaloids which have smaller pK_a values (<7.5) are likely to precipitate with II, while these which have larger pK_a values (>7.5) do not.

IR absorption of precipitates of quinine, papaverine or emetine with II at near 1750 and 1620 cm^{-1} show that they have two kinds of carboxylic acid moieties, ionical and nonionical, although they are precipitated at pH 3 where carboxylic acid moieties of free II exist in nonionic form[1] (Fig. 2).

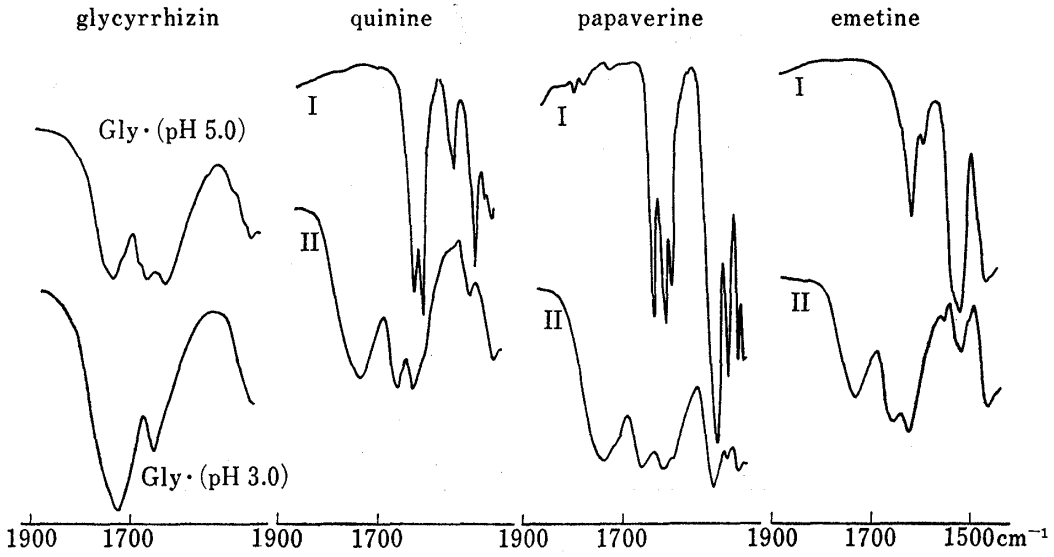

Fig. 2. IR Spectra of Alkaloids and Their Glycyrrhizinates

I: base, II: salt.

The content of noscapine (III) in its precipitate with II determined by UV absorption at 312 nm was shown to be 46.9%, while that of calculated value in which two mol of III bound with one mol of II was 50.1%. This result suggests that molar ratio of II and III in the precipitate is 2: 1. The content of tetrahydroberberine (IV) and corydaline (V) in the precipitate with II determined by HSLC method were 25.3 and 26.0%, while their calculated values were 29.2 and 31.0%. These data suggest that each one mol of IV or V bind with one mol of II in the precipitate. (Table II).

Table II. Determination of Noscapine, Tetrahydroberberine and Corydaline in Their Salts with Glycyrrhizin

	Cont. %	Cont. calcd. (Alk : Gly)[a]	Method
Noscapine	46.9	50.1(2 : 1)	UV
Tetrahydroberberine	25.3	29.2(1 : 1)	HSLC
Corydaline	26.0	31.0(1 : 1)	HSLC

a) Alkaloid: glycyrrhizin.

From the results mentioned above, it may be presumed that alkaloids and II bind ionically in the aqueous solution in different ratio, and form precipitate when their salt have small solubility in water.

Precipitation reaction, however, may be affected not only by the solubility or pK_a value of the alkaloid also by the solubility and the stability constant of the salt with II, so, further investigation should be necessary to clarify the mechanism of the precipitation phenomenon of this type.

II. Precipitation Reaction of Glycyrrhizin with Crude Drugs which contain Various Alkaloids in Aqueous Solution

Eleven kinds of crude drugs which contain alkaloids are used commonly in the 210 preparations which are certified by the Ministry of Health and Welfare Japan and there are more than 60 preparations in them which contain both glycyrrhiza radix and alkaloidal crude drugs. Accordingly, the precipitation reaction of II with these crude drugs was examined.

200 ml of distilled water is added on 3 g of crude drugs and decocted for 1.5 hr to the concentration of one-half of its original volume and filtered through cotton fiber. By addition of saturated solution of II (K salt), the decoction solutions made from coptis rhizome, phellodendron cortex, corydalis roots or evodia fruits produced a large quantity of precipitate, while those made from areca seeds, aconite roots and ephedra herb produced only a slight amount of precipitate, and those made from magnolia cortex, sinomenium roots or nupharis rhizome gave no precipitate. The same result was obtained by alkaloid fraction dissolved in a small quantity of EtOH and water which had been previously separated by extraction of the decoction solution with $CHCl_3$ under alkaline condition (Table III).

Table III. Precipitation Reaction of Glycyrrhizin with Crude Drugs which contain Alkaloids in Aqueous Solution

Name	Precip. react.[a]	No. prepn.[b]
Aconit tuber	±	1
Arecae semen	±	1
Coptidis rhizoma	+	15
Corydalis tuber	+	1
Ephedrae herba	±	17
Evodiae fructus	+	2
Magnoliae cortex	−	18
Nupharis rhizoma	−	1
Phellodendri cortex	+	7
Sinomeni caulis *et* rhizoma	−	3

a) Precipitation reaction.
b) Number of preparations which are certified by the Ministry of Health and Werfare Japan.

These results agree very closely with the results of the precipitation reaction of the simple alkaloid with II. The decoction solution of the crude drugs which contain alkaloids that do not precipitate, namely, ephedrine, aconitine, sinomenine do not give precipitate with II. Arecoline and related compounds in areca seeds and nuphar alkaloids may be predicted not to precipitate with II by considering the chemical structure and the pK_a value of them. On the other hand, crude drugs which contain berberine, corydaline and other berberine type alkaloids, namely, coptis rhizome, phellodendron cortex corydalis roots produced much precipitate. Evodia fruits which contain evodiamine and rutecarpine, that may be expected to precipitate with II, produce a large quantity of precipitate and the rough taste of the solution disappears at the same time.

Glycyrrhiza radix is widely used for the treatment of many other crude drugs (shuji, 修治) and said to eliminate the bitterness of preparations with the sweetness of II. However, it may also be assumed that precipitation reaction of II with bitter substances play a role

in disappearance of the bitterness or roughness in the preparations.

It has been already reported that the recovery of II in the preparations containing ephedra herb and glycyrrhiza radix showed lower than those without ephedra herb.[6] Though ephedrine does not precipitate with II in aqueous solution, it is suggested to form ion pair with II in the solution from the result of the specific conductance measurement (Fig. 3). So, we must pay much attention to the precipitation reaction or ion pair forming reaction in the decoction preparation of crude drug mixtures used in Kampoo, so that they may be responsible for both lowering of recovery and altering the pharmacological activities of the crude drugs contained in the various Kampoo preparations.

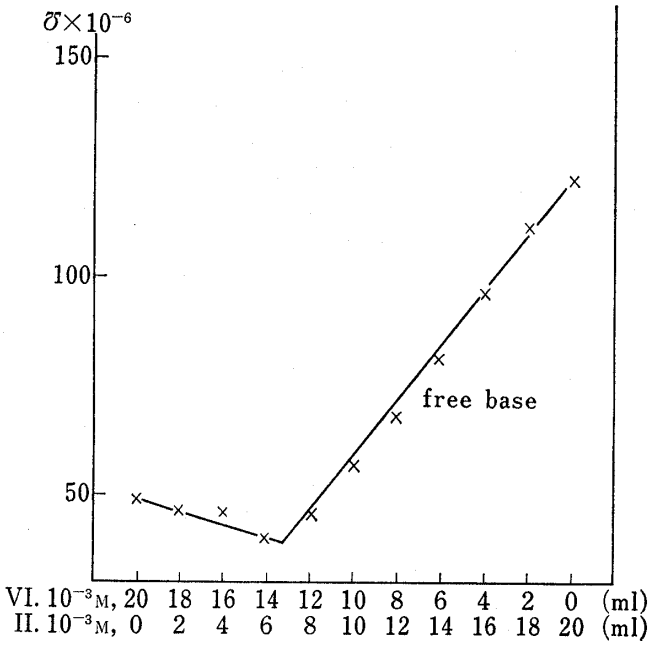

Fig. 3. The Specific Conductance of the Mixture of Ephedrine (VI) and Glycyrrhizin (II) Solutions

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6) M. Noguchi, *Kampoo Kenkyu*, **1978**, No. 4, 122.