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Studies on the Evaluation of Crude Drug. I. Quantitative Estimation of Anthraquinones in *Cassia* Seeds[1]

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A method of the quantitative estimation of anthraquinones in Ketsumeishi was established and the change of anthraquinone contents in the seeds of *Cassia obtusifolia* L. and *C. tora* L. with various grade of maturity and ageing was studied. Also, the quantitative estimations of anthraquinones in Ketsumeishi samples obtained from various places were made. The absorbance of the alkaline extract after treatment with 5% sodium hydroxide solution containing 2% ammonia was measured at 520 nm, using 1,8-dihydroxyanthraquinone as a standard. The optimum time for measurement was between 60 and 180 min after the completion of alkaline extraction and the optimum time for hydrolysis was 75 min. The recovery rate in the standard addition test was 97.0%. In the progress of maturity of the seeds, the amount of free anthraquinones decreased and the amount of bound anthraquinones increased gradually, although the total amount was constant. The contents of both free and bound anthraquinones of fresh seeds were lower than those of the seeds preserved for 1 to 9 years. In Ketsumeishi samples, the contents of free anthraquinones were 0.01—0.04%, those of bound anthraquinones 1.01—1.29%, and the total contents varied from 1.04 to 1.31%. It was suggested that the crude drugs obtained from different places might be classified into two groups according to the contents of anthraquinones.

Keywords——Ketsumeishi; *C. obtusifolia* L.; *C. tora* L.; 1,8-dihydroxyanthraquinone; evaluation of crude drugs; anthraquinone contents

The Chinese crude drug Juenmingzi (Ketsumeishi in Japanese) which is the seed of *Cassia obtusifolia* L. or *C. tora* L. (Leguminosae) has been used as a laxative and a tonic and also utilized as a substitute for tea in Japan. Some chemical studies have been made on the constituents of this crude drug. Anthraquinones isolated from the drug include emodin,[3] chrysophanol, physcion, aloe-emodin, rhein,[4a,b] obtusifolin,[4a] obtusin, chryso-obtusin, aurantio-obtusin,[5] and other compounds.[6] However the anthraquinone contents of this crude drug have not yet been estimated. In this study a method for the quantitative estimation of anthraquinones in Ketsumeishi was established, and the change of anthraquinone

1) This work was presented at the 97th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1977.
2) Location: *2–1, Oshika-2-chome, Shizuoka-shi, Shizuoka, 422, Japan.*
3) J. Shinomiya, *Apotheker Ztg.*, **11**, 537 (1896).
4) *a*) M. Takido, *Chem. Pharm. Bull.* (Tokyo), **6**, 397 (1958); *b*) T. Kariyone and K. Tsukita, *Yakugaku Zasshi*, **94**, 223, 225 (1954).
5) M. Takido, *Chem. Pharm. Bull.* (Tokyo), **8**, 264 (1960).

contents in the seeds of *C. obtusifolia* L. and *C. tora* L. with various grade of maturity and ageing was studied and the quantitative estimations of anthraquinones in Ketsumeishi obtained from various countries were made by using this method.

Experimental

Materials——1) Commercial Ketsumeishi Powder. 2) Ketsumeishi: Korea A, obtained in 1973; Korea B, obtained in 1974; China A, obtained from Shantung prefecture in 1973; China B, obtained from Hong-Kong in 1974; China C, obtained from Shantung prefecture in 1976; China D, obtained from Hong-Kong in 1976; Japan A1, produced in 1972; Japan A2, produced in 1973; Japan A3, produced in 1976; Japan B, produced in 1972; Japan C1, produced in 1975; Japan C2, produced in 1976; Viet-Nam A, obtained in 1974; Viet-Nam B, obtained in 1973. 3) Seeds of *Cassia tora* L.; Formosa A, produced in 1972; Formosa B, produced in 1967; Formosa C, produced in 1976. These materials were procured from drug markets in Osaka or produced in the plant garden of Shizuoka College of Pharmacy (Japan C) or yielded in the herb garden of Osaka University (Japan A). The Korean, Chinese and Japanese Ketsumeishi samples were identified as *C. obtusifolia* L. and the Vietnamese Ketsumeishi samples as *C. tora* L. Ketsumeishi obtained from various places were powdered and used for the experiments.

Apparatus——Hitachi spectrophotometer Model 124 was used for the determination of absorption maximum. The absorbance was measured by Shimadzu spectrophotometer Model UV 140.

Quantitative Estimation of Anthraquinones

As Ketsumeishi contains various anthraquinones, the separation and the quantitative estimation of each anthraquinone are very difficult. Since the alkaline solutions of different anthraquinones have similar absorption spectra and molar extinction coefficients,[7] 1,8-dihydroxyanthraquinone was used as a standard substance for estimating the concentrations of different anthraquinone glycosides. Thus, the amount of total anthraquinones was calculated as 1,8-dihydroxyanthraquinone.

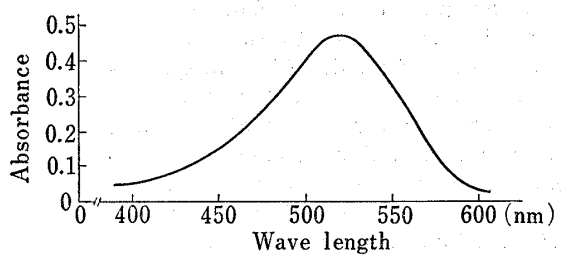

Fig. 1. Absorption Spectrum of 1,8-Dihydroxyanthraquinone

1,8-Dihydroxyanthraquinone239 μg/100 ml Alkaline solution (5% NaOH in 2% $NH_3 \cdot H_2O$)

Determination of the Absorption Maximum of the Standard Substance——239 μg of 1,8-dihydroxyanthraquinone was dissolved in 100 ml of 5% sodium hydroxide solution containing 2% ammonia.[8] The absorbance of the above solution was determined at the wave length ranging from 400 to 600 nm. The absorption maximum was observed at 520 nm (Fig. 1). The molar extinction coefficient of this solution was 1.2×10^4 at 520 nm.

Calibration Graph——A stock solution was prepared by dissolving 12.8 mg of 1,8-dihydroxyanthraquinone in 250 ml of 5% aqueous solution of sodium hydroxide containing 2% ammonia. The working dilutions 1 in 25, 2 in 25, 4 in 25, 6 in 25, 8 in 25 and 10 in 25 ml/ml were prepared and the absorbance of each dilution was measured at 520 nm. A linear relationship was obtained between the concentration (μg/ml) and the absorbance. The regression equation obtained: $Y=0.0503X+0.0017$, X=concentration (μg/ml), Y=absorbance.

Isolation of Free Anthraquinones——One gram of accurately weighed powdered drug was macerated with 30 ml of ether for 24 hours. The ether extract was separated by filtration and the residue was extracted further with ether several times till all the free anthraquinones were extracted (as judged by the colour of the ether). The ether extracts were combined and preserved for the estimation of free anthraquinones (extract I).

Isolation of Anthraquinone O-Glycosides——The marc that remained after the extraction of free anthraquinones was refluxed with 2 ml of concentrated hydrochloric acid and 15 ml of glacial acetic acid on a water bath. After cooling the mixture, 30 ml of ether was added and refluxing was continued. The ether/acid mixture was filtered into a separater and the marc was further boiled with 30 ml of ether. The combined ether extract was washed with distilled water to remove excess of acid and was used for the estimation of anthraquinone O-glycosides (extract II).

Estimation of Anthraquinones in Ether Extracts I and II——The ether extracts I and II were treated with a sufficient volume of aqueous 5% sodium hydroxide solution containing 2% ammonia till the ether

6) R.P. Tiwari and JaiRajBehari, *Planta Medica*, **21**, 393 (1972); M. Kaneda, E. Morishita, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **17**, 458 (1969).
7) Summary Report of Technical Researches in the Health and Welfare Administration, 1976.
8) K.H. Müller and B.G. Christ, *Arch. Pharm.*, **295**, 41 (1962); German Pharmacopoeia DAB7, 1306.

layer became colourless (alkaline extracts I and II). The alkaline extract I was collected in 100 ml-volumetric flask and was made up to 100 ml with the alkaline solution. The alkaline extract II was collected in 250 ml-volumetric flask and the final volume was adjusted to 250 ml with the alkaline solution. Ten ml of the above solution was diluted to 25 ml in a volumetric flask with the alkaline solution. The absorbance of each solution was measured at 520 nm.

Results and Discussion

Stability of the Alkaline Extract

The absorbance of the alkaline extract tended to increase with time for 60 min, reaching an equilibrium state which lasted for about 2 hours, and then showed a gradual decrease (Fig. 2). Therefore, the optimum time for measurement is between 60 min and 180 min after the completion of alkaline extraction. The time required for reaching an equilibrium state seems to depend on the reactivities of the anthraquinones and the alkaline solution.

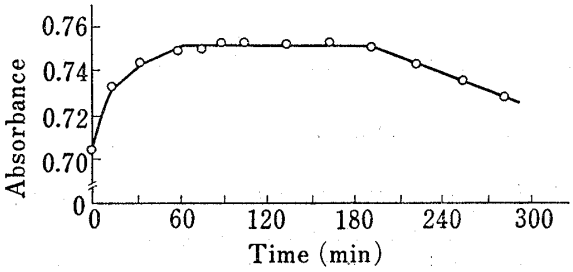

Fig. 2. Stability of Alkaline Extract

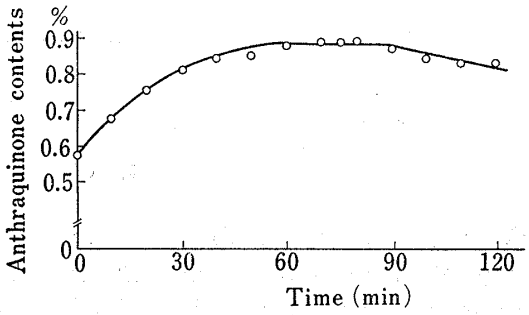

Fig. 3. Change of Anthraquinone Amounts with Time of Hydrolysis

Change of Anthraquinone Amounts with Time of Hydrolysis

The extractable anthraquinone amount changed with the time of hydrolysis and the amount increased with time for 60 min, reaching the highest level which lasted for 90 min, and then decreased gradually (Fig. 3). Thus, the optimum time for hydrolysis appears to be 75 min.

Table I. Precision and Recovery Rates in Standard Addition Test

Sample	Observed value %						Av.	C.V. %
Drug	0.90	0.87	0.88	0.90	0.88	0.93	0.89	2.46

No. of Exp.	Added amount of standard (mg)	Amount recovered (mg)	Recovery %
1	10.20	9.89	97.0
2	17.50	16.81	96.1
3	17.80	17.45	98.0
4	14.14	13.67	96.7
	Recovery average		97.0
	C.V. %		0.85

Precision and Recovery Rates in Standard Addition Test

For the examination of this quantitative estimation method, the quantitative analysis of Ketsumeishi powder was repeated six times. The average of total anthraquinone contents

was 0.86% and the coefficient of variation was 2.46%. For the examination of recovery rates, 1,8-dihydroxyanthraquinone was added to Ketsumeishi powder and the quantitative analysis was repeated for four times. The average recovery rate in the standard addition test was 97.0% (Table I). The results show that this method can be used satisfactorily for the quantitative analysis of Ketsumeishi.

Change of Anthraquinone Contents in the Seeds of *Cassia obtusifolia* L. and *C. tora* L. with Various Grade of Maturity and Ageing

C. obtusifolia and *C. tora* plants flower in August and set seeds in September in Japan. The seeds grow in the legume and the colour of the seeds changes with the progress of maturity as shown in Fig. 4. When the seeds become mature, the colour of the seed coat becomes

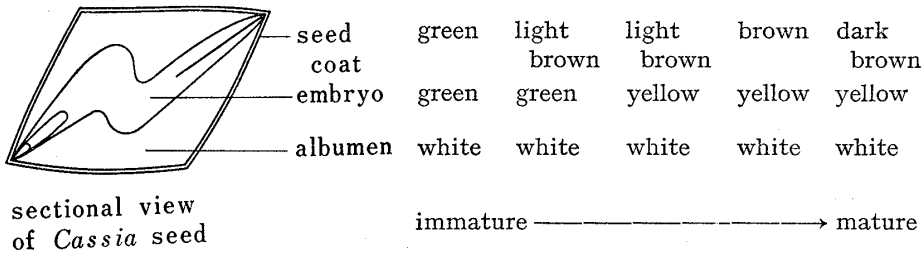

Fig. 4. Change of the Colour of *Cassia* Seed

dark brown and the embryo becomes yellow and the legume bursts open. Although the change of anthraquinone contents in the seeds with various grade of maturity and ageing has not been made clear, the following results were obtained in this study. In the progress of maturity of the seeds, the content of free anthraquinones decreased and that of bound anthraquinones increased gradually, although the total amounts remained constant (about 0.85%). However, when the fresh seeds DB(C)-Y(E)[9] were compared with the seeds preserved for 1 to 9 years after harvest, the anthraquinone contents of the former were smaller than those of the latter which were about 1.1% irrespective of age (Table II). The reason for the apparent increase in the anthraquinone content during seed storage is not clear. Therefore

Table II. Change of Anthraquinone Contents among the Seeds of *C. obtusifolia* L. and *C. tora* L. with Various Grade of Maturity and Ageing

Samples[a)]	Maturity ageing	Free %	Bound %	Total %	C.V. %
Japan A	G(C)—G(E)	0.12	0.68	0.80	2.50
	LB(C)—G(E)	0.10	0.76	0.86	2.42
	LB(C)—Y(E)	0.06	0.78	0.84	3.38
	B(C)—Y(E)	0.01	0.84	0.85	1.66
	DB(C)—Y(E)	0.02	0.84	0.86	2.27
	1 year old	0.02	1.12	1.14	1.75
Japan C	DB(C)—Y(E)	Trace	0.95	0.95	1.49
	2 year old	0.02	1.07	1.09	1.30
	4 year old	0.02	1.09	1.10	
Formosan	DB(C)—Y(E)	Trace	0.84	0.84	2.02
	4 year old	0.03	1.06	1.09	
	9 year old	0.03	1.01	1.04	

G: green, LB: light brown, DB: dark brown, B: brown, Y: yellow, (C): seed coat, (E): embryo.
Immature G(C)—G(E)——DB(C)—Y(E) mature
a) The Japanese Ketsumeishi samples were identified as *C. obtusifolia* L. and the Formosan Ketsumeishi samples as *C. tora* L.

9) DB(C): colour of the seed coat, DB: dark brown, Y(E): colour of the embryo, Y: yellow, See Fig. 4.

the seeds which are over one year after harvest should be used for the quantitative estimation of anthraquinones.

Contents of Anthraquinones in Crude Drugs

The quantitative estimations of Ketsumeishi produced in various countries were made. The results are shown in Table III. The anthraquinones were present both in the free and in the bound state in all the samples analyzed. The contents varied from 0.01 to 0.04% for free anthraquinones, 1.01 to 1.29% for bound anthraquinones, and 1.04 to 1.31% for total anthraquinones. Based on the contents of anthraquinones (Table III), these samples of Ketsumeishi may be classified into two groups: one group consisted of Korean Ketsumeishi, China A and China C which show an anthraquinone content higher than 1.20% and the other group including China B, China D, Japanese, Vietnamese and Formosan Ketsumeishi containing less than 1.10% anthraquinones. Although the anthraquinones contents were different among the crude drugs obtained from various places, no significant difference was found between *C. obtusifolia* and *C. tora* and further studies are needed to decide the relationship between these groups and the two species.

Table III. Contents of Anthraquinones in Crude Drugs

Samples[a)]	Free %	Bound %	Total %	C.V. %
Korea A	0.02	1.21	1.23	2.70
Korea B	0.03	1.20	1.23	1.15
China A	0.04	1.18	1.22	1.50
China C	0.02	1.29	1.31	4.25
China B	0.02	1.09	1.11	—
China D	0.02	1.02	1.04	1.47
Japan A1	0.01	1.09	1.10	—
Japan A2	0.02	1.07	1.09	1.30
Japan B	0.01	1.09	1.10	1.57
Japan Cl	0.02	1.12	1.14	1.75
Viet-Nam A	0.01	1.03	1.04	1.76
Viet-Nam B	0.02	1.08	1.10	1.82
Formosa A	0.03	1.06	1.09	—
Formosa B	0.03	1.01	1.04	—

a) The Korean, Chinese and Japanese Ketsumeishi samples were identified as *C. obtusifolia* L. and Vietnamese and Formosan as *C. tora* L. The quantitative analysis of Ketsumeishi powder was repeated from four to six timeis.

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