[Chem. Pharm. Bull. 26(8)2458—2464(1978)]

UDC 615.322.011.5.015.1.076.9 : 543.544.2.062

Quantitative Analysis of the Purgative Components of Rhubarb and Senna

Haruji Oshio, Yoshitomo Naruse,[1a] and Makoto Tsukui[1b]

Pharmacognostic Research Laboratory, Takeda Chemical Industries, Ltd.[1]

(Received February 18, 1978)

A study on the estimation of sennosides and derivatives of oxyanthraquinones in rhubarb and senna is described.

After extracting a ground crude drug with the mixture of calcium acetate buffer and tetrahydrofuran (THF), the extract was fractionated to aqueous and THF phases. Both fractions were heated with sulfuric acid to hydrolyze the glycosides contained, and the resulting aglycones were extracted with ether. The aqueous fraction containing sennidins and rhein was analyzed by spectrophotometry. The THF fraction containing only oxyanthraquinones was analyzed by densitometry on a thin-layer plate.

It was suggested that sennosides were the main purgative principles of rhubarb or senna, and that oxyanthraquinones had little contribution to the activities of crude drugs.

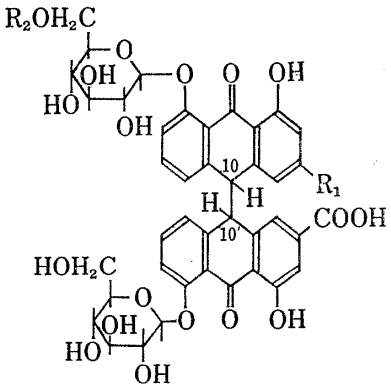
The qualities of rhubarbs varied more markedly than those of sennas. It was clarified that the activities of crude drugs in mice correlated closely to the contents of sennosides. One can, therefore, estimate the purgative activity roughly from the contents of sennosides.

Keywords——rhubarb; senna; sennoside; oxyanthraquinone; photodensitometry; densitometric chromatoscanning; purgative activity; active components-activity correlation

Rhubarb, "Rhei Rhizoma (大黄)," the rhizome of *Rheum* spp. (Polygonaceae), has been used as an excellent purgative crude drug in Chinese medicine. Senna, "Sennae Folium (センナ)," the leaf of *Cassia angustifolia* or *C. acutifolia* (Leguminosae), is also a well-known purgative crude drug.

A number of reports[2] described the estimation of the purgative compounds such as sennosides and oxyanthraquinones in the both drugs. But those assays seemed to be not

Table I. Structures and Purgative Activities of Sennosides



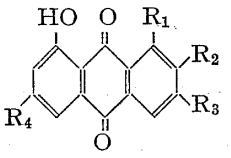
	R_1	R_2	10–10′	ED_{50}[a]	Origin[b]
Sennoside A	COOH	H	*threo*	13.5	S, R
B	COOH	H	*erythro*	13.9	S, R
C	CH_2OH	H	*thereo*	13.3	S, R
D	CH_2OH	H	*erythro*	15.8	S, R
E	COOH	OC-COOH	*threo*	13.5	R
F	COOH	OC-COOH	*erythro*	16.1	R

a) ED_{50} shows purgative activity in mice (mg/kg).
b) S; senna, R; rhubarb.

1) Location: *a*) *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan*; *b*) *Ichijoji-Takenouchi-cho, Sakyo-ku, Kyoto 606, Japan.*

2) *a*) R. Dequeker, J. Lemli, and J. Cuveele, *Planta Med.*, **12**, 51 (1964); *b*) J. Lemli, *J. Pharm. Pharmacol.*, **17**, 227 (1965); *c*) G. Richter and I.H. Hauenstein, *Deut. Apoth.-Ztg.*, **107**, 1751 (1967); *d*) Y. Asahi, K. Shinozaki, M. Mitani, and H. Ohtsuka, *Chem. Pharm. Bull.* (Tokyo), **22**, 254 (1974); *e*) Y. Ogihara, O. Inoue, H. Otsuka, K. Kawai, T. Tanimura, and S. Shibata, *J. Chromatogr.*, **128**, 218 (1976).

Table II. Free Oxyanthraquiones from Rhubarb or Senna



	R_1	R_2	R_3	R_4
Chrysophanol	OH	H	CH_3	H
Physcion	OH	H	CH_3	OCH_3
Emodin	OH	H	CH_3	OH
Aloe-emodin	OH	H	CH_2OH	H
Citreorosein	OH	H	CH_2OH	OH
Rhein	OH	H	COOH	H
Laccaic acid D	CH_3	COOH	OH	OH

Table III. Purgative Activities of Oxyanthraquinones

	ED_{50} (mg/kg)
Chrysophanol	>500
Physcion	>500
Emodin	>500
Aloe-emodin	59.6
8-Glu. aloe-emodin	71.6
Rhein	97.5
8-Glu. rhein	103.0

applicable to estimating the quality of a crude drug owing to imprecision or complicacy of the analytical methods. We attempted effective separation of active components followed by acid hydrolysis and quantitative determinations of the resulting aglycones by densitometry.

This paper deals with a quantitative analysis of active components, sennosides, rhein, aloe-emodin, emodin, physcion and chrysophanol. The correlation between purgative activity and content of active components of rhubarb or senna is also discussed.

Experimental[3]

Materials——Rhubarbs were obtained from markets in Japan, Hong Kong, Thailand and Germany except Shin-Shu Daio (a hybrid between *R. coreanum* and *R. palmatum*[4]) which was cultivated in Hokkaido for 4 or 5 years. Sennas were obtained commercially from Tochimoto Tenkaido Co. Ltd. (Osaka).

The ground crude drugs (*ca.* 100 mesh) were dried over silica gel for 2 weeks at room temp. before analysis.

Bioassay——The purgative activity in mice was estimated by the method of the authors and expressed as ED_{50} value (mg/kg).[5]

Quantitative Analyses of Sennosides and Oxyanthraquinones——About 50 mg of material was weighed accurately, and suspended in 15 ml of calcium acetate buffer[6] for 30 min. To the aqueous suspension, 30 ml of tetrahydrofuran (THF) was added and the mixture was stirred for additional 30 min. After filtration, the residue was extracted again with 5 ml of a mixture of the buffer and THF (1 : 2). The extracts were combined, and shaken vigorously with 9 g of NaCl for 30 min to make saturation. When the mixture separated to 2 layers, it was divided into aqueous (Fr. 1) and THF (Fr. 2) phases. Fr. 2 was extracted again with 10 ml of saturated aqueous NaCl, and the aqueous extract was combined to Fr. 1.

(1) After adding 3.5 ml of 97% H_2SO_4 slowly, Fr. 1 was heated in a boiling water-bath for 25 min without reflux condenser. The reaction solution was extracted twice with ether (50, 30 ml), and the combined ether extracts were washed with 10 ml of water. After evaporation of the ether, the residue was dissolved in 50 ml of 2 N KOH solution.

3) Spectrophotometer: Hitachi Perkin-Elmer 139 Type S 12–8, Chromatoscanner: Shimadzu Dual-Wavelength TLC Scanner CS-910.
4) T. Matsuoka and R. Hatta, *J. Takeda Res. Lab.*, **29**, 776 (1970).
5) M. Tsukui, M. Yamazaki, Y. Toyosato, T. Matsuoka, and H. Fujimura, *Yakugaku Zasshi*, **94**, 1095 (1974).
6) Calcium acetate buffer: the pH of 1 N $Ca(CH_3COO)_2$ solution was adjusted to 5.3 with CH_3COOH, and the solution was diluted 10-fold volume with distilled water.

The absorbances of the alkaline solution at 398 (T_1) and 503 nm (T_2) were measured with a spectrophotometer.

$$\text{Sennosides (g)} = S_1 \times \frac{1}{374.1} \times \frac{862.72}{538.44} \times \frac{50}{100} = 2.1454\,T_1 - 0.3411\,T_2$$

$$\text{8-Glycosylrheins (g)} = R_2 \times \frac{1}{335.6} \times \frac{446.35}{284.21} \times \frac{50}{100} = 2.3445\,T_2 - 0.0305\,T_1$$

Where 862.72, 538.44, 446.35 and 284.21 correspond to the molecular weights of sennoside A, sennidin B, 8-glucosylrhein and rhein, respectively.

(2) After adding 10 ml of 10% H_2SO_4, Fr. 2 was heated in a boiling water-bath for 30 min without reflux condenser. The reaction solution was extracted three times with ether (50, 20, 20 ml), and the combined ether extracts were washed with 10 ml of water. After evaporation of the ether, the residue was dissolved in 5 ml of THF.

An aliquot of the THF solution was spotted on a thin-layer plate [Silica gel $60F_{254}$ (Merck); thickness 0.25 mm]. The plate was first developed in hexane–THF–HCOOH (130: 10: 0.1) to a height of *ca.* 15 cm. The absorbance of the spots at *Rf* 0.32 (chrysophanol) and 0.23 (physcion) were examined with a densitometric chromatoscanner. Then the plate was developed again in benzene–HCOOEt–AcOEt–HCOOH (75: 24: 0.8: 0.2), and the absorbances at *Rf* 0.40 (emodin) and 0.30 (aloe-emodin) examined individually. The plate was then developed in $CHCl_3$–THF (5: 1), and absorbance at *Rf* 0.30 (rhein) was examined.

Scanning: transmitted beam, zig-zag scanning, linearlizer Ch. 1, $\lambda_R = 700$ nm, $\lambda_S = 425$ nm (chrysophanol), 430 nm (physicon), 435 nm (emodin), 425 nm (aloe-emodin) and 430 nm (rhein). The conditions of the apparatus were checked beforehand by azobenzene ($\lambda_S = 425$ nm).

Results and Discussion

The purgative action on rhubarb and senna had been ascribed to oxyanthraquinone until sennosides A and B, the stereoisomers of 8,8′-diglucosylrhein dianthrone, were isolated from senna by Stoll *et al.*[7] in 1949. Sennosides C and D were also isolated later from the same drug.[8] Zwaving,[9] on the other hand, first detected sennosides A, B and C by paper chromatography of the constituents of *R. palmatum* in 1965. And six analogs of sennosides A, B, C, D, E and F, have been isolated up to now from rhubarbs.[10] Lemli *et al.*[2a,11] and Saber *et al.*[12] isolated free oxydianthrones as the minor components of rhubarb and leaf and pod of *C. obovate*.

The well-known oxyanthraquinones in rhubarb and senna are rhein, aloe-emodin, emodin, physcion, chrysophanol and their glycosides.[10c,d,13] Recently, the authors[10d] isolated two free oxyanthraquinones, citreorosein and laccaic acid D, and two glucosides of the latter from rhubarb.

Although it is desirable to determine the contents of all purgative components for quality estimation of a crude drug, it is practically impossible, because about thirty analogs of oxyanthraquinones or oxydianthrones are contained in the crude drugs. Fortunately, the contributions of the free oxydianthrones, citreorosein and laccaic acid D to purgative activity will be negligible because of their poor contents. All sennosides revealed almost comparable activities in a bioassay with mice (Table I). The activities of oxyanthraquinones widely varied

7) A. Stoll, B. Becker, and W. Kussmaul, *Helv. Chim. Acta*, **32**, 1892 (1949).
8) W. Schmid and E. Angeliker, *Helv. Chim. Acta*, **48**, 1911 (1965); J. Lemli and J. Cuveele, *Pharm. Acta Helv.*, **40**, 667 (1965).
9) J.H. Zwaving, *Planta Med.*, **13**, 474 (1965).
10) *a*) M. Miyamoto, S. Imai, M. Shinohara, S. Fujioka, M. Goto, T. Matsuoka, and H. Fujimura, *Yakugaku Zasshi*, **87**, 1040 (1967); *b*) J.H. Zwaving, *J. Chromatogr.*, **35**, 562 (1968); *c*) H. Oshio, S. Imai, S. Fujioka, T. Sugawara, M. Miyamoto, and M. Tsukui, *Chem. Pharm. Bull.* (Tokyo), **22**, 823 (1974); *d*) H. Oshio, *Shoyakugaku Zasshi*, **32**, 19 (1978).
11) J. Lemli, R. Dequeker, and J. Cuveele, *Planta Med.*, **12**, 107 (1964).
12) A.H. Saber, S.I. Bulbaa, and A.T. Awad, *Lloydia*, **25**, 238 (1962).
13) J.H. Gardner, *J. Am. Pharm. Ass.*, **28**, 143 (1939); H. Wagner, L. Hörhammer, and L. Farkas, *Z. Naturforsch.*, **186**, 89 (1963); L. Hörhammer, G. Bittner, and H.P. Hörhammer, *Naturwissenschaften*, **51**, 310 (1964); H. Okabe, K. Matsuo, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **21**, 1254 (1973).

individually. But the activities between rhein or aloe-emodin and their corresponding glucosides were practically the same (Table III).

Thus it is reasonable that the contents of all sennosides can be determined *en bloc*, but those of rhein, aloe-emodin, emodin, physcion and chrysophanol should be determined individually after hydrolyzing the glycosides to estimate the quality of rhubarb or senna.

The active components were extracted almost quantitatively with a mixture of calcium acetate buffer and THF from powder of the crude drug. By saturating with sodium chloride, the extract was separated into two layers. The lower aqueous layer (Fr. 1) contained sennosides, 8-glucosylrhein and 8-(oxalyl-) glucosylrhein. The upper layer (Fr. 2) contained the other oxyanthraquinones (Fig. 1).

(1) Sennosides and rhein glycosides were hydrolyzed to sennidins (95.85% yield), the aglycones of sennosides, and rhein (100.00% yield), respectively by heating Fr. 1 with aqueous sulfuric acid.

Both sennosides A and E are hydrolyzed to sennidin A, and sennosides B and F to sennidin B. Sennidin A isomerizes readily to sennidin B in an alkaline solution. In the same manner sennidin D is derived from sennosides C and D. Sennidins B and D show the absorption maxima at 398 and 397 nm, respectively. Since sennosides A and B are major components of rhubarb or senna, the optical density at 398 nm was measured for determination of the amount of sennidins *en bloc* in a photometric analysis.

A content of sennosides can be calculated from the amount of sennidins as that of sennoside A, because the molecular weight of the sennoside is comparable to each other.

An alkaline solution of rhein shows the absorption maximum at 503 nm.

Therefore, by determining the absorbances at 398 and 503 nm of an alkaline solution of the mixture of sennidin B and rhein, the real absorbance of each compound is defined as follows:

$$S_1 = \frac{T_1 - k_2 T_2}{1 - k_1 k_2} = \frac{T_1 - 0.159\,T_2}{0.998},$$

$$R_2 = \frac{T_2 - k_1 T_1}{1 - k_1 k_2} = \frac{T_2 - 0.013\,T_1}{0.998}$$

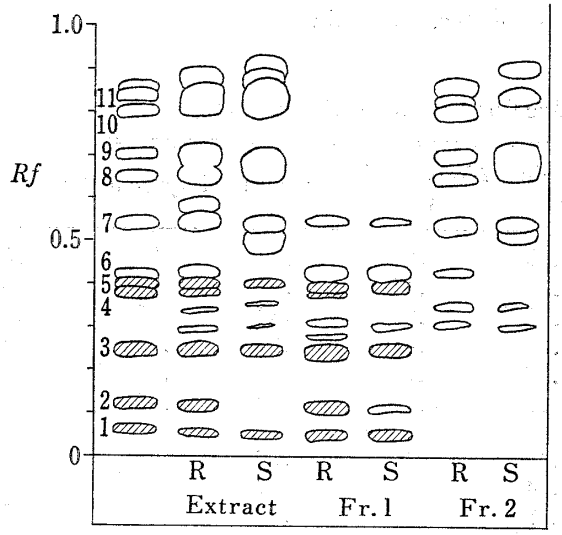


Fig. 1. TLC of the Constituents of Rhubarb and Senna

Adsorbent: Silica gel 60 F_{254} (Merck), solvent: AcOEt–PrOH–H_2O (4: 4: 3), detection: 5% KOH/EtOH.
R; rhubarb, S; senna. Authentics: 1 sennoside B, 2 sennoside E, 3 sennoside A, 4 sennoside D, 5 sennoside C, 6 8-glucosylrhein, 7 rhein, 8 8-glucosylemodin, 9 8-glucosyl-aloe-emodin, 10 aloe-emodin, 11 emodin.

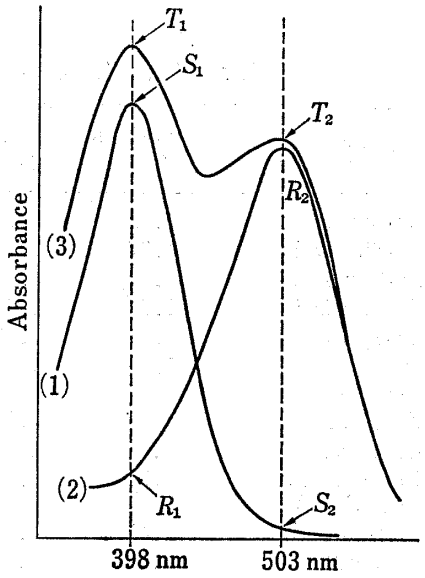


Fig. 2. Absorptions of Sennidin B and Rhein in 2 N KOH

(1) Sennidin B, (2) rhein, (3) mixture of sennidin B and rhein.
$E_{1\%}$: S_1; 374.1, S_2; 4.7, R_1; 53.5, R_2; 335.6.

where S_1 is absorbance of sennidin B at 398 nm, R_2 absorbance of rhein at 503 nm, T_1 and T_2 absorbance of mixture at 398 and 503 nm, respectively, and k_1 and k_2 constants. k_1 and k_2 are defined as follows:

$$k_1 = \frac{E_{1\%} \text{ of sennidin B at 503 nm}}{E_{1\%} \text{ of sennidin B at 398 nm}} = \frac{4.7}{374.1} = 0.013,$$

$$k_2 = \frac{E_{1\%} \text{ of rhein at 398 nm}}{E_{1\%} \text{ of rhein at 503 nm}} = \frac{53.5}{335.6} = 0.159$$

The sennosides and 8-glycosylrheins contents of a rhubarb, Shin-Shu Daio (No. 4), for example, were determined as 2.26% (C. V. 4.53%, n=6) and 1.60 (7.4, 6) respectively. After adding sennoside A and 8-glucosylrhein, the rhubarb was examined again. The recoveries of each compound were 94.8% (C. V. 2.94%, n=5) and 97.1 (5.12, 5) respectively.

(2) Oxyanthraquinone glycosides were also hydrolysable quantitatively by heating Fr. 2 with aqueous sulfuric acid. The separation of free oxyanthraquinones was carried out on a thin-layer plate precoated with silica gel. The absorbance at the absorption maximum of anthraquinone was measured with a densitometric chromatoscanner, and the amount was determined from a calibration curve (Fig. 3).

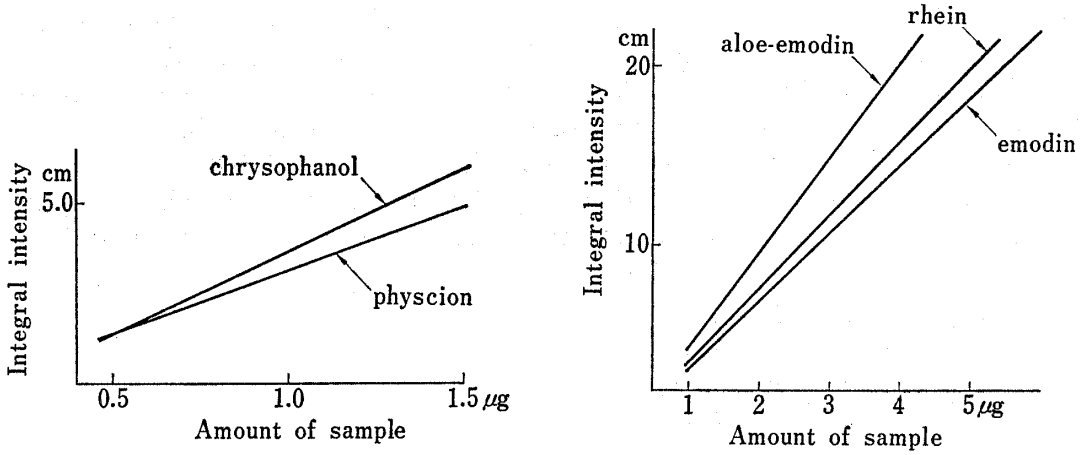


Fig. 3. Calibration Curves of Oxyanthraquinones

Table IV. Contents of Purgative Components of Rhubarbs (%)

	Origin	Sennosides (x)	Glu. rhein (a_1)	Rhein (a_2)	Aloe-emodin (a_3)	Emodin	Physcion	Chrysophanol
1	Shin-Shu Daio	5.62	2.21	0.35	0.93	0.36	0.27	0.57
2	Shin-Shu Daio	4.97	1.96	0.35	0.41	0.35	0.26	0.67
3	Shin-Shu Daio	2.30	1.48	0.31	0.39	0.30	0.25	0.50
4	Shin-Shu Daio	2.26	1.60	0.19	0.48	0.29	0.25	0.35
5	Shin-Shu Daio	1.84	1.47	0.15	0.26	0.20	0.22	0.37
6	Gao (Hong Kong)	1.84	1.91	0.29	0.18	0.20	0.05	0.22
7	Shin-Shu Daio	1.77	1.14	0.43	0.44	0.33	0.29	0.62
8	(Germany)	1.69	2.20	0.24	0.23	0.11	0.04	0.23
9	Kinmon Daio (Hong Kong)	1.51	1.29	0.23	0.35	0.42	0.18	0.79
10	Shin-Shu Daio	1.41	1.28	0.18	0.27	0.27	0.19	0.31
11	(Thailand)	1.36	1.57	0.26	0.18	0.23	0.11	0.18
12	Kinmon Daio (Japan)	0.85	0.83	0.12	0.16	0.23	0.17	0.51
13	(Germany)	0.84	0.73	0.24	0.16	0.23	0.17	0.32
14	Batei Daio (Hong Kong)	0.61	0.51	0.11	0.44	0.13	0.19	0.39

The rhein, aloe-emodin, emodin, physcion and chrysophanol contents of a rhubarb, Shin-Shu Daio (No. 4), were determined as 0.19% (C. V. 5.21%, $n=5$), 0.48 (4.23, 5), 0.29 (3.56, 5), 0.25 (8.29, 5) and 0.35 (7.18, 5) respectively. When the authentic compounds were added to the rhubarb (No. 4) and examined again, 95.6% (C. V. 5.19%, $n=5$) of 8-glucosyl-aloe-emodin and 99.2% (2.15, 5) of 8-glucosylemodin were recovered.

Free oxyanthraquinones were recovered almost quantitatively.

1. From Table I, III, IV and V, it was suggested that sennosides were the main purgative principles of rhubarb or senna, and oxyanthraquinones have little contribution to the activity. The quality of rhubarbs varied much more than those of sennas.

Table V. Contents of Purgative Components of Sennas (%)[a)]

	Commercial grade	Sennosides (x)	Glu. rhein	Rhein	Aloe-emodin	Emodin
1	Grade 3	2.67	0.39	0.17	0.08	Trace
2		2.44	0.38	0.12	0.06	0.02
3	Grade 1	2.30	0.37	0.24	0.07	Trace
4	Grade 2	2.19	0.33	0.15	0.07	Trace

a) All the drugs examined contained little of physcion and chrysophanol

2. Activity, in general, corresponds with contents of active principles. In the case of rhubarb, it was proved by examining a correlation between the contents of sennosides (x) and the activities ($1/ED_{50}\times10^3=y$) (Table IV and VI): correlation efficient; $r=0.975$, $p<0.01$. An equation of regression curve was defined as:

$$y = 2.36\,x + 0.64 \quad (2<y<15) \tag{1}$$

Although activities of oxyanthraquinones were of lower level than those of sennosides (Table III), the derivatives of rhein and aloe–emodin seemed to contribute a little to the activity. The contributions of those compounds, therefore, were added to those of sennosides after rectifying the contents of the formers as follows:

$$f_1 = a_1 \times 13.5/103.0 = 0.13\,a_1 \text{ (8-glucosylrhein)}$$
$$f_2 = a_2 \times 13.5/97.5 = 0.14\,a_2 \text{ (rhein)}$$
$$f_3 = a_3 \times 13.5/59.6 = 0.23\,a_3 \text{ (aloe-emodin)}$$
$$x' = x + f_1 + f_2 + f_3$$

where x' represents the rectified total contents of sennosides and the oxyanthraquinones (%). Correlation efficient; $r=0.976$, $p<0.01$. An equation of regression curve was defined as:

$$y = 2.17\,x' + 0.37 \quad (2<y<15) \tag{2}$$

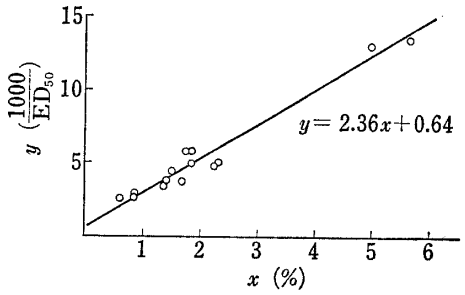


Fig. 4. Correlation between Purgative Activity and Content of Sennosides in Rhubarb

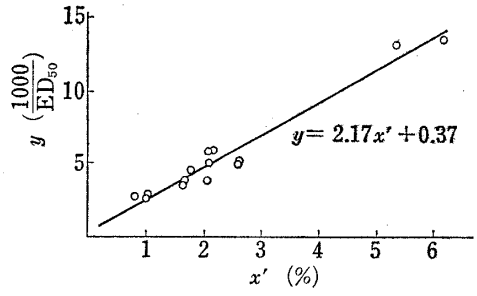


Fig. 5. Correlation between Purgative Activity and Total Content of the Active Components in Rhubarb

The calculated ED_{50} values from the equation (1) or (2) practically agreed to the observed ones except several samples (Table VI).

Table VI. Observed and Calculated Purgative Activities of Rhubarbs (ED_{50}; mg/kg)

Sample No.	Observed	Calcd. (eq. 1)	Calcd. (eq. 2)
1	73	72(68— 76)[a]	73(68— 78)[a]
2	76	81(76— 86)	83(78— 90)
3	196	165(146—189)	165(144—193)
4	207	168(148—193)	166(145—194)
5	200	201(174—238)	202(172—246)
6	170	201(174—238)	197(168—238)
7	171	207(179—248)	205(174—250)
8	169	216(185—260)	207(175—253)
9	222	238(201—292)	235(195—337)
10	263	252(211—313)	251(205—322)
11	290	260(216—326)	254(208—328)
12	338	377(292—535)	388(289—588)
13	388	382(294—543)	394(292—602)
14	379	481(350—796)	474(334—813)

a) 95% Confidence limits are given in parentheses.

Now we can conclude that the purgative activity of rhubarb can be estimated by analysis of the contents of the active components, especially that of sennosides.

3. We could not employ enough samples to discuss a correlation between contents of active components and the purgative activity of senna.

However, the activity of senna may be estimated roughly from the contents of sennosides, because a correlation between sennoside contents and ED_{50} values of senna will also be in an inverse proportion like rhubarb. The ED_{50} value (y) of senna are calculated by the following equation:

$$xy = A \qquad (3)$$

Where x represents the content of sennosides and $A=\sum_{i=1}^{4} x_i y_i/4=729$ ($\sigma=63$) (Tables V, VII).

Table VII. Observed and Calculated Purgative Activities of Sennas (ED_{50}; mg/kg)

Sample No.	Observed (y)	Calcd. (eq. 3)
1	272	273(226—320)
2	400	299(247—350)
3	298	317(262—372)
4	241	333(275—390)