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Investigation of Rhubarbs. III.[1] New Purgative Constituents, Sennosides E and F

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Besides sennoside A, sennosides B, C, E and F were isolated from rhubarbs. Sennosides E and F are new compounds and have comparable purgative activity to other sennosides. Sennosides E and F are stereoisomer mutually, and they are oxalates of sennosides A and B, respectively. Although sennoside A was able to be isomerized to sennoside B irreversibly, the plant contained more of the former.

Rhubarbs are excellent purgative crude drugs, and their constituents have been investigated for a long time. The famous constituents of rhubarbs are oxyanthraquinones; chrysophanic acid,[3] emodin,[4] aloe-emodin,[5] physcion,[6] rhein[7] and their glycosides[8] (Table I). Fairbairn[9] and Matsuoka[10] concluded that oxyanthraquinones are less active than glycosides.

On the other hand, in 1965, Zwaving[11] found sennosides A, B and C as the constituents of rhyzome of *Rheum palmatum* L. on a paper partition chromatogram (Table II). Then Miyamoto, *et al.*[12] succeeded in isolating sennoside A from rhubarbs.

We noticed an interesting fact that strong activity remained in the mother solution, which excluded sennoside A by the method of Miyamoto. Moreover, three distinct spots, similar to sennoside A, were visible as blue black spots under an ultraviolet ray on a Lumi color plate (Wako) on a paper partition chromatogram of the mother solution. The *Rf* values of them were 0.60 (**1**), 0.37 (**2**), 0.24 (**3**) and 0.18 (**4**), respectively.[18] Compound (**2**) was identical with sennoside A. But the other compounds could not be identified exactly. The ground rhubarbs were added with saturated sodium chloride aqueous solution and tetrahydrofuran. The brown organic solution contained slight object substances. The muddy residue was acidified with oxalic acid, then re-extracted with tetrahydrofuran. The extracted organic solution was concentrated to dryness, after having been passed through the column packed with Polyamide C-200 (Wako). The black mass of extracts was dissolved in methyl alcohol.

1) Part II: H. Oshio, S. Imai, S. Fujioka, T. Sugawara, M. Miyamoto and M. Tsukui, *Chem. Pharm. Bull.* (Tokyo), **20**, 621 (1971).
2) Location: *a*) *Juso-nishino-cho, Higashiyodogawa-ku, Osaka*; *b*) *Ichijoji-takenouchi-cho, Sakyo-ku, Kyoto.*
3) R. Brandes, *Ann. Chem.*, **9**, 85 (1834); J. Shlossberger and O. Dopping, *ibid.*, **50**, 196 (1844); F. Gstirner and H. Haltzen, *Pharmazie*, **4**, 333 (1949).
4) R. Segal, I. Milo-Galdzweig and D.D. Zaitschek, *Lloydia*, **27**, 237 (1964); K. Tsukida, *Yakugaku Zasshi*, **74**, 386 (1954); S. Shibata and M. Takido, *ibid.*, **72**, 1311 (1952).
5) M. Uchibayashi and T. Matsuoka, *Chem. Pharm. Bull.* (Tokyo), **9**, 234 (1961).
6) K. Tsukida, N. Suzaki and M. Yokota, *Yakugaku Zasshi*, **74**, 224 (1954).
7) E. Schratz, *Planta Med.*, **8**, 301 (1960).
8) L. Hörhammer, G. Bittern and H.P. Hörhammer, *Naturwissenshaften*, **51**, 310 (1964).
9) J.W. Fairbairn, C.A. Friedman and H.A. Ryan, *J. Pharm. Pharmacol.*, **10**, 1867 (1958).
10) T. Matsuoka, *Shoyakugaku Zasshi*, **15**, 113 (1961).
11) J.H. Zwaving, *Planta Med.*, **13**, 474 (1965).
12) M. Miyamoto, S. Imai, M. Shinohara, S. Fujioka, M. Goto, T. Matsuoka and H. Fujimura, *Yakugaku Zasshi*, **87**, 1040 (1967).

Table I. Anthraquinones from Rhubarbs

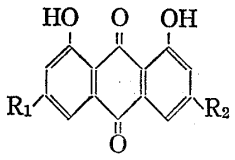
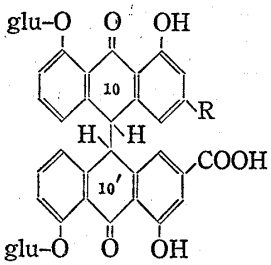
		R_1	R_2
	Chrysophanic acid	H	CH_3
	Physcion	OCH_3	CH_3
	Emodin	OH	CH_3
	Aloe-emodin	H	CH_2OH
	Rhein	H	COOH

Table II. Structure of Sennosides A, B, C and D

		R	10—10′
	Sennoside A	COOH	*trans*
	Sennoside B	COOH	*meso*
	Sennoside C	CH_2OH	*trans*
	Sennoside D	CH_2OH	*meso*

After three days, the yellow powders were precipitated and they were recrystallized from acetone–water (7: 3). The yellow crystals were sennoside A.

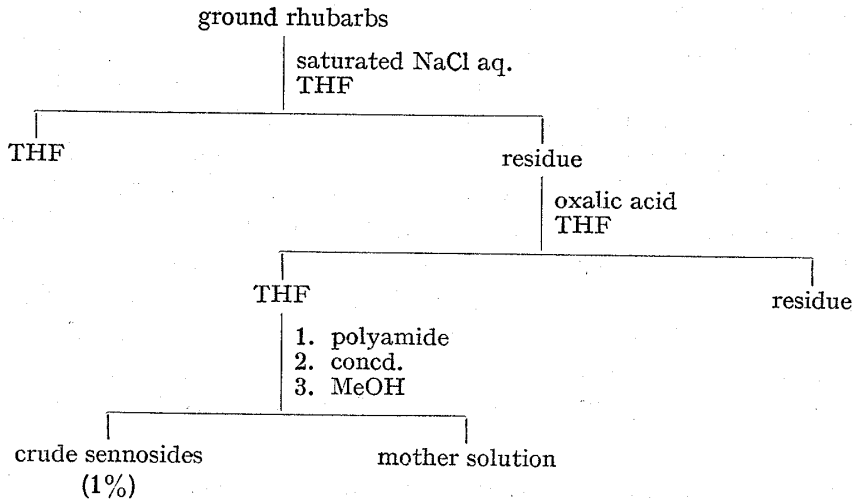


Chart 1. Isolation of Crude Sennosides from Rhubarbs

As compounds (**1**), (**3**) and sennoside A remained in the mother solution, they were separated on a column of Sephadex LH-20 by the method of Zwaving,[13] and recrystallized from acetone–water (7: 3) and afforded yellow needles respectively.

On the other hand, the mother solution, excluding the yellow powders, was chromatographed on a column of silica gel which was washed with 2M hydrochloric acid previously. After thorough washing with ethyl acetate, the active fraction was eluted by acetone–water (99: 1). It was rechromatographed twice by the method of Zwaving, thus compound (**4**) and a new active compound (**5**) were isolated. The *Rf* values of the latter was 0.11 on the paper partition chromatogram same as the above system.

13) J.H. Zwaving, *J. Chromatogr.*, **35**, 562 (1968).

Table III. Sennosides Isolated from Rhubarbs

	mp	*Rf* on PPC	Yield (%)
Compound (1) (sennoside C)	204—206	0.60	0.038
Compound (2) (sennoside A)	220—243 (decomp.)	0.37	0.772
Compound (3) (sennoside E)	214—215	0.24	0.067
Compound (4) (sennoside B)	209—212	0.18	0.010
Compound (5) (sennoside F)	171—175 (decomp.)	0.11	0.001

PPC: Toyo-roshi No. 51, *n*-BuOH–EtOH–0.2M citrate buffer (pH 6.2) (2: 1: 2), ascending method

Compound (**1**) afforded yellow needles from acetone–water (7: 3); mp 204—206°, $[\alpha]_D$ −128.1° (c=0.2 acetone–water (7: 3)). It had, similar to sennoside A, maximum ultraviolet absorptions at 270 and 323 nm in 0.5% sodium bicarbonate solution. The infra red spectrum of compound (**1**) resembled sennoside A, but the intensity of absorption at 1704 cm^{-1}, referred to absorption of carboxyl groups, was less than that of sennoside A. When treated with potassium permanganate, compound (**1**) was changed to sennoside A. It was easily assumed that compound (**1**) was identical with sennoside C, although the reported melting point was higher (Table IV).[14] As shown in Table IV, it was identified by direct comparison with the authentic sample of sennoside C.[15] The infrared (IR) spectra of both compounds were also identical with each other.

Table IV. Comparison of Compounds (**1**) and (**4**) with Sennosides C and B

	mp	*Rf* on PPC	λ_{max} (nm)	$[\alpha]_D$
Compound (**1**)	204—206	0.60	270, 323	−128.1
Sennoside C	214—216	0.60	270, 323	−123
Compound (**4**)	209—212	0.18	270, 308, 355	− 93
Sennoside B	180—186	0.18	270, 308, 355	−100

Compound (**4**) afforded yellow needles from acetone-water (7: 3); mp 209—212°, $[\alpha]_D$−93° (c=0.2 acetone–water (7: 3)). It had maximum ultraviolet absorption at 270, 308 and 355 nm in 0.5% sodium bicarbonate solution, and the same pattern as sennoside B or D.[16] It was also identified by direct comparison with the authentic sample of sennoside B (Table IV).[15]

Compound (**3**) also gave fine yellow needles from acetone–water (7: 3); mp 214—215°, $[\alpha]_D$−122.8° (c=0.2, acetone–water (7: 3)). The infrared and ultraviolet (UV) spectra of compound (**3**) revealed very similar patterns of absorption to that of sennoside A (Fig. 1 and Fig. 2). Compound (**3**) was not identical with sennoside D, the known fourth sennoside, for pattern the of the ultraviolet spectrum of the latter was similar to sennoside B.[16]

The patterns of optical rotatory dispersion (ORD) and circular dichromism (CD) curves of compound (**3**) were also very similar to sennoside A (Fig. 3 and Fig. 4).

Now it was supposed that compound (**3**) belonged to sennosides, and was the fifth new one, so we named this compound "sennoside E". It was easily assumed that sennoside E was a derivative of sennoside A, because the former transferred to the latter slowly in acetone–water (7: 3). This reaction was accelerated by adding a base, for example, sodium bicarbonate (Fig. 5).

14) W. Schmid and E. Angeliker, *Helv. Chim. Acta*, **48**, 1911 (1965).
15) We are in debt to Drs. H.V. Balthasar and H. Friedli to offer the samples of sennosides B and C.
16) J. Lemli and J. Cuveele, *Pharm. Acta Helv.*, **40**, 667 (1965).

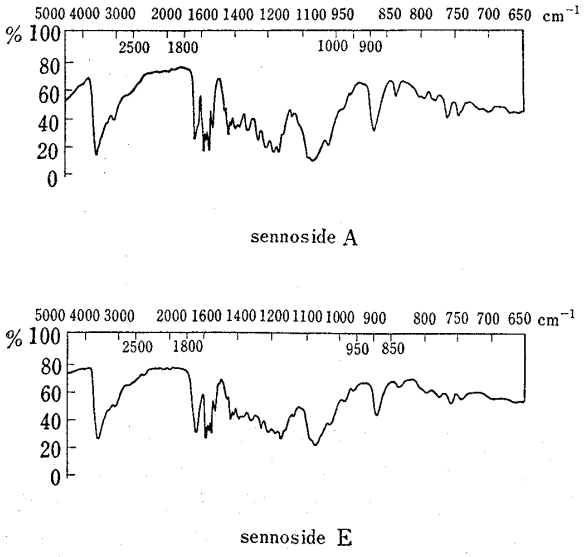


Fig. 1. IR Spectra of Sennosides A and E (KBr disk)

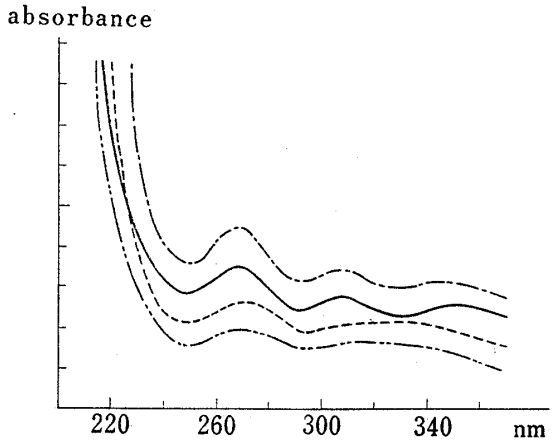


Fig. 2. UV Spectra of Sennosides A, B, E and F in 0.5% $NaHCO_3$ Solution

——: sennoside A
——: sennoside B
– – – –: sennoside E
– – – –: sennoside F

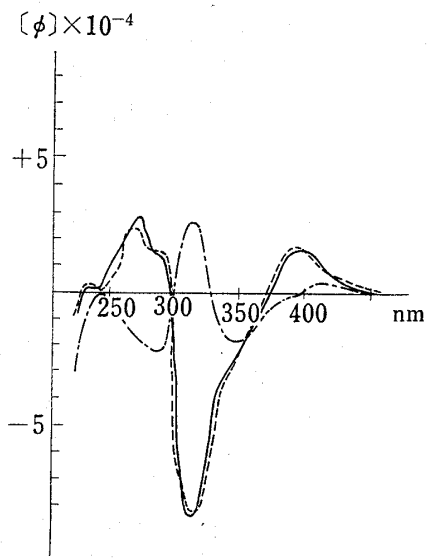


Fig. 3. ORD Curves of Sennosides A, B and E in Dioxane–Water (7 : 3)

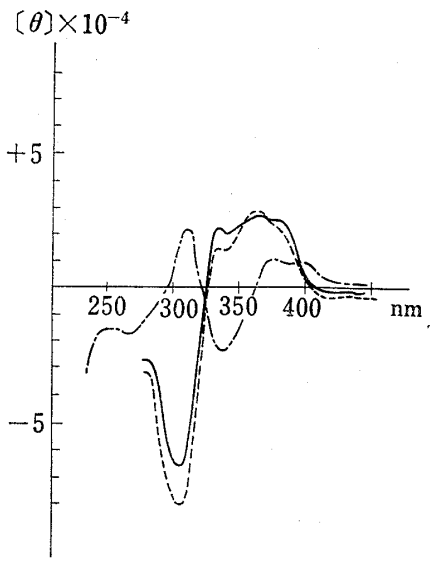
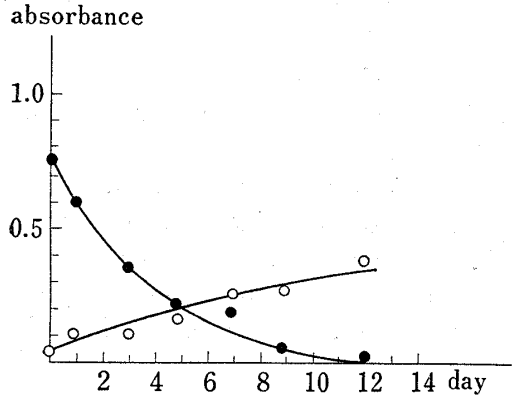
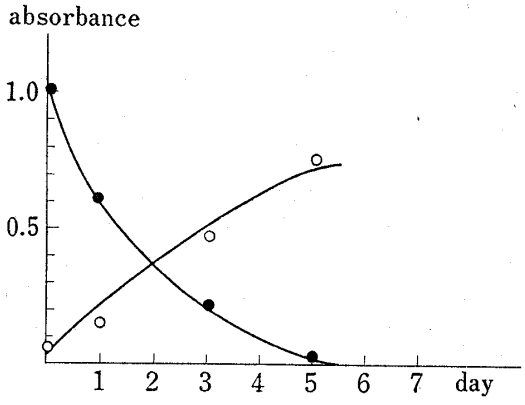


Fig. 4. CD Curves of Sennosides A, B and E in Dioxane–Water (7 : 3)

——: sennoside A
– – – –: sennoside B
——: sennoside E



(a) In 0.5% $NaHCO_3$ solution



(b) In 10% $NaHCO_3$ solution

Fig. 5. Transformation of Sennosides E to A

●: decrease of sennoside E, ○: increase of sennoside A

The pertrimethyl siloxy derivatives of sennosides A and E revealed almost the same pattern in mass spectra. In both compounds, the most detected mass was namely *m/e* 863, corresponding to M/2 of pertrimethyl siloxy sennoside A. This means, perhaps, that sennoside E was changed to sennoside A on the way to silylation. It was assumed that a dianthrone would be cleaved to two anthrones easily, and the most present mass would correspond to half of the molecule.

Both sennosides A and E were hydrolyzed and afforded one mole of aglycone, sennidin A, and two moles of glucose by incubation with β-glucosidase from snails or heating in mineral acid solution. When sennoside E was reduced by sodium hydrosulfite,[17] sennosides A, B, E, compound (**5**), 8-glucosylrhein and an unknown compound (**6**) were observed on a paper partition chromatogram, although the forecasted anthrones were not isolated.

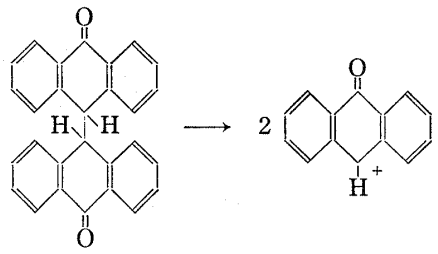


Chart 2

While sennoside A afforded 8-glucosylrhein anthrone by reduction, also sennosides A, B and 8-glucosylrhein were visible on a paper partition chromatogram of the latter. It was suggested that sennoside E cleaved to 8-glucosylrhein anthrone and another anthrone, then they were changed to six kinds of compounds by oxidation during chromatography. It was important for the elucidation of structure that compound (**5**) was derived from sennoside E. The new compound (**6**) turned violet when sprayed with an alkaline solution as 8-glucosylrhein, thus suggesting an anthraquinone.

When sennoside E was oxidized by *m*-chloroperbenzoic acid directly, 8-glucosylrhein and compound (**6**) were isolated. Compound (**6**) was orange yellow powder, mp 170—172°, and its ultraviolet absorption spectrum was similar to 8-glucosylrhein. But the intensity of absorption at 1730 cm^{-1} was stronger in compound (**6**) than in 8-glucosylrhein in the infra red spectra.

In a basic solution, compound (**6**) was easily changed to 8-glucosylrhein as sennoside E to A. It was interesting that compound (**6**) was also isolated from rhubarbs as a constituent.

For neutralization of sennoside E or compound (**6**), three or two moles of sodium hydroxide were used. This meant that sennoside E or compound (**6**) had one more carboxylic group than sennoside A or 8-glucosylrhein. Then oxalic acid was identified after saponification of both sennoside E and compound (**6**). Both compounds, therefore, are oxalates of sennoside A or 8-glucosylrhein respectively, and one of carboxyl groups in oxalic acid remains free.

Since the phenolic hydroxyl groups of both sennoside E and compound (**6**) were observed near δ 12 ppm on nuclear magnetic resonance (NMR) spectra, the position of ester was in sugar moiety. If the phenolic hydroxyl group was blocked, the ultraviolet absorption spectra should not be comparable to both 8-glucosylrhein and compound (**6**).

For the instability, the position of ester was not elucidated exactly. It was concluded that the structure of compound (**6**) is 8-(oxalyl)glucosyl-3-carboxylyl-1-hydroxyanthraquinone, and that of sennoside E is 8-glucosyl-8′-(oxalyl)glucosyl-3,3′-dicarboxyl-1,1′-dihydroxydianthrone (10—10′ *trans*).

Compound (**5**) was yellow powder; mp 171—175° (decomp.), $[\alpha]_D$ −48.7° (c=0.2, acetone–water (7: 3)), and an extremely minor constituent of rhubarbs. The pattern of ultraviolet absorption spectrum belonged to the type of sennoside B (Fig. 2). But compound (**5**) was not identical with sennoside D, because it was more hydrophobic than sennoside B, and could be derived from sennoside E as described above. Thus we named compound (**5**) "sennoside F."

Sennoside F was unstable in atmospheric conditions, and decomposed to sennoside B slowly. For the instability and low yield of sennoside F, we could not get adequate informa-

17) A. Stoll, B. Becker and A. Helfenstein, *Helv. Chim. Acta*, 33, 313 (1950).

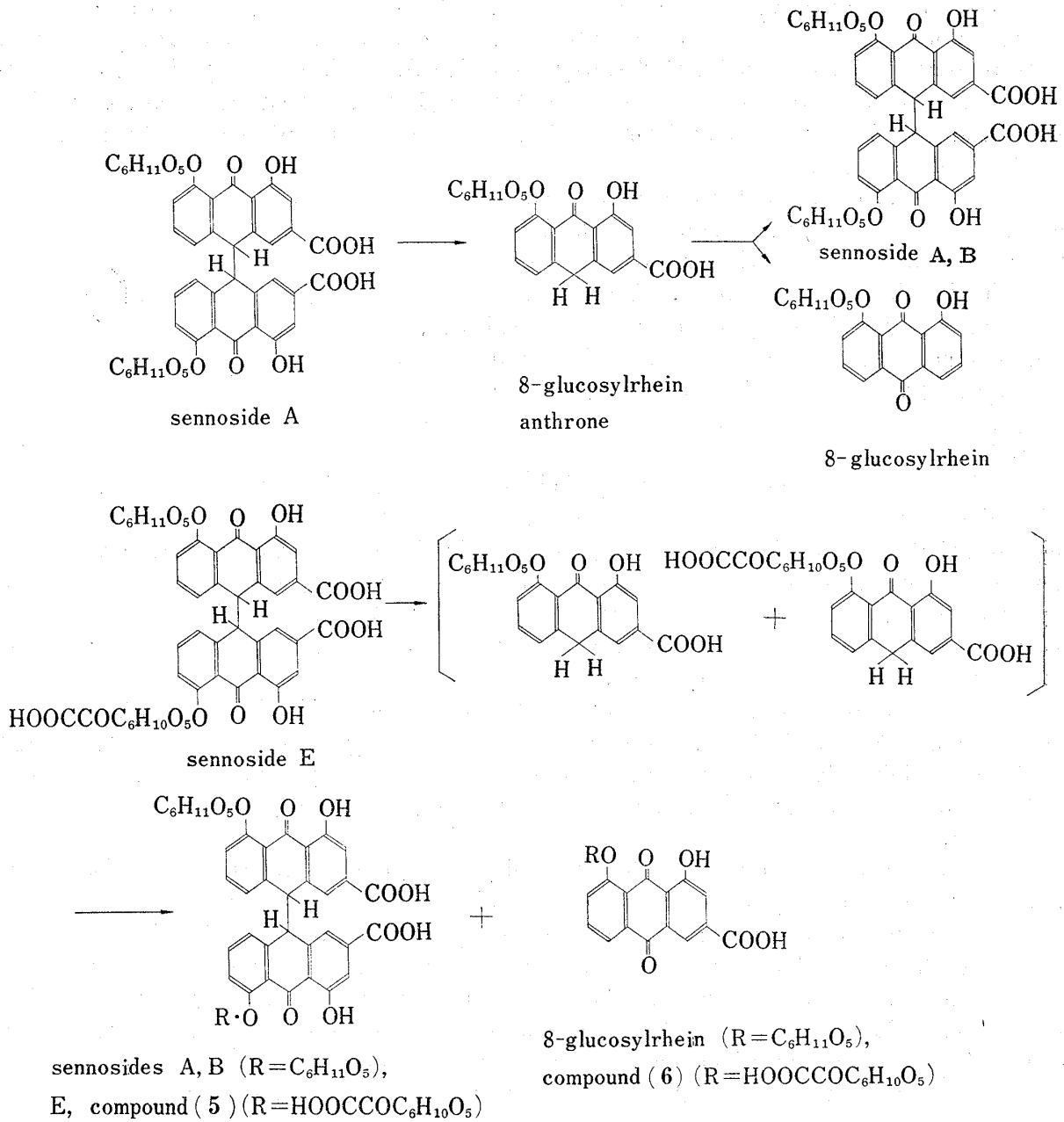


Chart 3

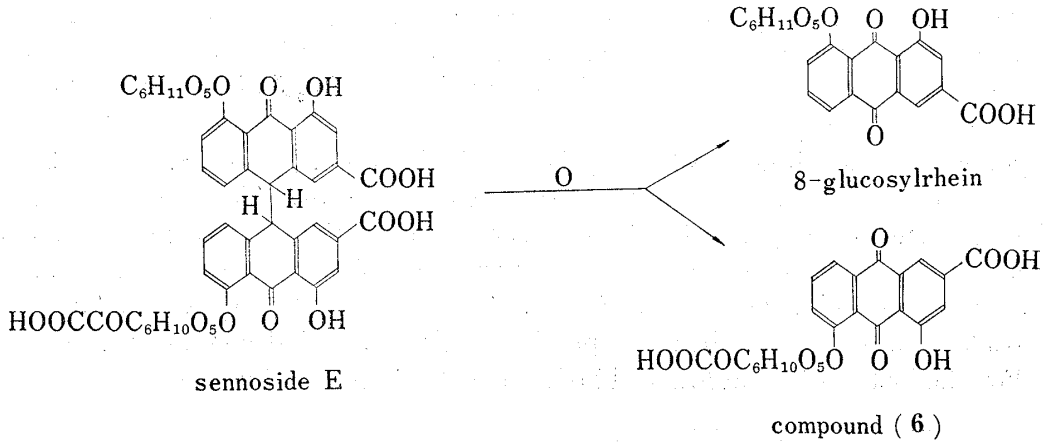
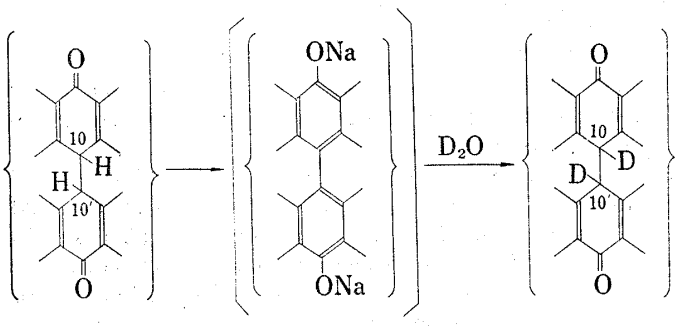


Chart 4

tion. It seemed, however, to be proper that sennoside F is the 10—10′ *meso* typed stereoisomer of sennoside E. Because sennoside F also oxidized to 8-glucosylrhein and 8-(oxalyl)-glucosylrhein by *m*-chloroperbenzoic acid, and it could be derived from sennoside E as discussed above.

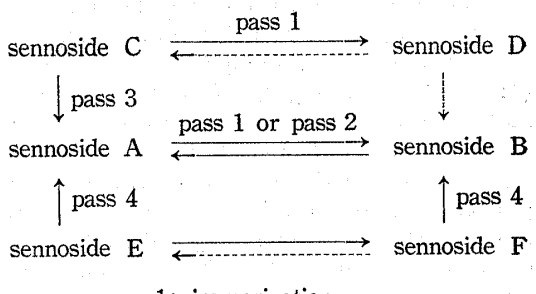
Sennoside A isomerized to B in sodium bicarbonate solution at 80° slowly, and the rate of reaction was acceralated by increasing of base. This reaction could be traced by using deuterium oxide and sodium carbonate, and observing the chemical shift at δ 6.51 ppm, corresponding to the protons at 10 and 10′ of sennosides, on the NMR. The intensity of the signal at 6.51 ppm decreased as isomerization proceeded, thus the mechanism of this reaction was assumed as follows.



sennoside A
(10—10′ *trans*)

deuterized sennoside B
(10—10′ *meso*)

Chart 5



pass 1: isomerization
pass 2: reduction and oxidation
pass 3: oxidation
pass 4: hydrolysis

Chart 6

Now it has been confirmed that six kinds of sennosides isolated from plants may be related chemically.

It is interesting that rhubarbs contain more *trans* isomer than *meso* isomer, although the former seems to be more unstable than the latter. On the other hand, senna, a purgative crude drug, contains almost equal amounts of both isomers.[17]

In rhubarbs, the sennosides seem to be produced through the different pass way from senna. So it is assumable that there is a proper mechanism to synthesize *trans* isomers when anthrones change to dianthrone by oxidative coupling.

The oxalates of sennosides or 8-glucosylrhein was detected on a paper partition chromatogram (PPC) of water extract of rhubarbs, so it was proved that oxalates were contained in the plant.

Experimental[18]

Isolation of Sennosides A, B, C, E and F——The mixture of 490 g of ground rhubarbs (Shinshu Daio) and 1 liter of saturated NaCl solution was washed twice with 2 liters of tetrahydrofuran (THF). The muddy residue was acidified with 50 g of oxalic acid and extracted three times with 2 liters of THF. The organic solution was dried over Na_2SO_4, then passed through the column packed with 200 g of Polyamide C-200 (Wako), then concentrated *in vacuo* to dryness. The dark brown residue was dissolved in 50 ml of MeOH, then 4.9 g of yellow powder (Fraction 1) were precipitated after three days. The mother solution was concentrated to dryness and afforded 52 g of dark brown mass (Fraction 2). Fraction 1 (16.4 g) was recrystallized from acetone–water (7: 3) and afforded sennoside A (8.7 g). The mother solution was concentrated *in vacuo* and gave 7.0 g of yellow brown powder. The powder was suspended in 200 ml of MeOH–water (1: 1), then neutralized with saturated $NaHCO_3$ solution, and chromatographed on a column of 900 g of Sephadex LH-20 (80 × 800 mm). Eluting by MeOH–water (7: 3), five fractions were obtained; each fractions

18) All melting points are not corrected.
PPC for sennosides and anthraquinones; Toyo-roshi No. 51, *n*-BuOH–EtOH–0.2M citrate buffer (pH 6.2) (ratio of 2: 1: 2), ascending method.

were acidified with HCl to pH 1.5, then extracted with *n*-BuOH, and concentrated *in vacuo* under 50°. The first fraction (0.364 g) contained sennosides B, E and F; ratio of *ca.* 1: 5: 1, the second did sennoside E (1.1 g), the third did sennoside A (3.7 g), the fourth did 8-glucosylrhein (0.55 g), and the fifth did sennoside C. Fraction 2 (159 g) was chromatographed on a column of silica gel (Merck Art. 7734), washed with 2M HCl previously, (100×800 mm). The active compounds were eluted from the column with acetone–water (99: 1) after thorough washing with 4 liters of AcOEt. They were rechromatographed twice on a column of Sephadex LH-20, then 70 mg of sennoside B and 12 mg of sennoside F were isolated. Each sennoside was characterized in Table III.

Isolation of 8-Glucosylrhein and 8-(Oxalyl)glucosylrhein——Fraction 2 (50 g) in above item was dissolved in 200 ml of MeOH, and made alkaline with 5% $Ba(OH)_2 \cdot 8H_2O$ MeOH solution. The violet precipitate was filtered and washed with AcOH–MeOH (pH 4.5), then extracted with 10% oxalic acid MeOH solution. The solution was concentrated *in vacuo*. The residue was dissolved in dilute $NaHCO_3$ solution, then chromatographed twice on a column of Sephadex LH-20 (30×60 mm), and 120 mg of 8-(oxalyl)glucosylrhein and 390 mg of 8-glucosylrhein were eluted from the column with water.

8-Glucosylrhein; yellow powder, mp 255—279° (decomp.).

8-(Oxalyl)glucosylrhein; orange yellow powder, mp 170—172°.

Oxidation of Sennoside C to A——About 1 mg of sennoside C was dissolved in 0.5 ml of acetone–water (6: 4). After adding 0.1 ml of 0.1N $KMnO_4$ solution, it was stirred for 48 hr. On a paper partition chromatogram of the reaction mixture, the spot corresponding to sennoside A was visible; *Rf* 0.37.

Hydrolysis of Sennoside E——(a) After dissolved in 0.855 ml of 0.5% or 10% $NaHCO_3$ solution, 11.0 mg of sennoside E were left in darkness at room temperature. The reaction mixtures were taken out 20 μl each, after the certain days, then sennoside A was separated from E by paper electrophoresis; Toyo roshi No. 51, 0.04M citrate buffer (pH 6.2), 900 V, 1.6 mA/cm, 1 hr. Zones corresponding to both sennosides were extracted with 0.5% $NaHCO_3$ solution, then the amounts of sennosides were determined by intensities of absorption at 270 nm. It was assumed that ε of both compounds was 18500.

(b) Sennoside E (80 mg) was dissolved in 4 ml of 10% $NaHCO_3$ solution and left in darkness for 5 days. After acidifying with dil. HCl solution, the solution was extracted twice with 10 ml of *n*-BuOH, and concentrated *in vacuo*. The yellow residue was recrystallized twice from acetone–water (7: 3), and gave 13 mg of yellow needles, which was identified with sennoside A by IR spectrum and PPC.

Hydrolysis of 8-(Oxalyl)glucosylrhein——The solution of 20 mg of 8-(oxalyl)glucosylrhein in 3 ml of 1% Na_2CO_3 solution was kept in darkness for 2 days. The red solution was extracted with AcOEt four times, after acidified with HCl. The extract was concentrated *in vacuo* and the residue was recrystallized from MeOH, and afforded 14 mg of yellow crystals. mp 255—278° (decomp.), its IR spectrum was identical with that of 8-glucosylrhein.

Identification of Oxalic Acid from Sennoside E or 8-(Oxalyl)glucosylrhein——Sennoside E or 8-(oxalyl)-glucosylrhein were hydrolized with 5% KOH solution in darkness over night. The solutions were passed through the column of Amberlite CG-120. Eluted oxalic acid was identified by PPCs.

	PhOH–water–HCOOH (75:25:1)	Ether–AcOH–water (13:3:1)
Acid from sennoside E	0.20	0.12
Acid from 8-(oxalyl) glucosylrhein	0.20	0.11
Oxalic acid	0.20	0.11

Whatman No. 1, ascending method, colorized with BPB.

Hydrolysis of Sennoside F——(a) Sennoside F was stocked in darkness at room temperature for 5 days, then it was changed to sennoside B completely on PPC, then recrystallized from water. mp 209—212°, its IR spectrum was identical with sennoside B.

(b) Small amount of sennoside F was dissolved in 0.5% $NaHCO_3$ solution, and left in darkness for 3 days. Sennoside B was proved on a PPC of reaction solution.

Hydrolysis of Sennoside E by β-Glucosidase——Sennoside E was incubated with β-glucosidase from snails in 0.3M phosphate buffer (pH 6.12) at 37° over night. From the reaction mixture, glucose was detected on a PPC (*Rf* 0.22); Toyo-roshi No. 51, ascending method, *n*-BuOH–AcOH–water (ratio of 4: 1: 5).

Quantitative Analysis of Glucose from Sennoside E——Sennoside E (21.8 mg) was dissolved in 0.3 ml of dil. $NaHCO_3$ solution, then added with 0.3 ml of 8N H_2SO_4 solution and heated at 100° for 2 hr. The precipitated yellow aglycone (10 mg) was filtered off, and the filtrate was neutralized with $BaCO_3$ and the white precipitate was filtered off again, then the colorless filtrate was diluted to 40 ml exactly. By the method of Somogyi, 1.97 moles of glucose was titrated.

Titrations of Sennoside E and 8-(Oxalyl)glucosylrhein——Samples of both compounds were titrated in the solution of MCS–water (2: 1) at 27° with 0.5N NaOH solution. Sennoside E digested 2.7 moles of base, and 8-(oxalyl)glucosylrhein did 2.0 moles.

Reduction of Sennoside E with $Na_2S_2O_4$——Sennoside E (1.0 g) was dissolved in 20 ml of 0.1N $NaHCO_3$ solution, then stirred in oil bath between 95—98° and added with 1 g of $Na_2S_2O_4$. After 10 min, additional 1 g of $Na_2S_2O_4$ was added, and heated for 20 min. After cooling, the reaction mixture was worked up as Stoll,[17] but the forecasted anthrones were not isolated. On the other hand, many spots corresponding to sennosides A, B, E, F, 8-glucosylrhein and 8-(oxalyl)glucosylrhein were detected on a PPC of reaction mixture.

Oxidation of Sennoside E by *m*-Chloroperbenzoic Acid——The solution of 170 mg of sennoside E and 769 mg of *m*-chloroperbenzoic acid in 10 ml of dioxane was heated at 90° for 9 hr. Almost equal amounts of 8-glucosylrhein and 8-(oxalyl)glucosylrhein were observed from the spots on PPC, then the reaction mixture was diluted with 100 ml of 1% $NaHCO_3$ solution. After stirring for 30 min, *m*-chlorobenzoic acid was filtered off, and the solution was extracted three times with 50 ml of *n*-BuOH, after acidified with HCl. Orange red powder was obtained from organic solution by concentration *in vacuo* under 50°. The powder was dissolved in a small amount of dil. $NaHCO_3$ solution, then chromatographed on a column of Sephadex LH-20, and 13 mg of 8-glucosylrhein, mp 253—280° (decomp.) and 20 mg of 8-(oxalyl)glucosylrhein, mp 169—171°, were eluted from the column by water.

Isomerization of Sennosides A to B——(a) A solution of 1 g of sennoside A in 100 ml of 0.5% $NaHCO_3$ solution was heated at 85° for 6.5 hr under bubbling of N_2. The solution was lyophylized, then chromatographed on a column of 200 g of Sephadex LH-20. The yellow substance was eluted from the column by water, and recrystallized from acetone–water (7 : 3) and afforded yellow needles, mp 209—211°, $[\alpha]_D$ −91.3° (c=0.13 acetone–water (7 : 3)). It was identical with sennoside B on a PPC.

(b) Each 100 mg of sennoside A were dissolved in 3.9 ml, 7.8 ml and 15.6 ml of 0.5% $NaHCO_3$ solutions respectively, and heated between 80—82° under bubbling of N_2. After 10 hr, the amounts of sennoside B were determined by PPC and OD. They were 49.0%, 77.1% and 68.8% to the initial amounts of sennoside A. In the last case the reaction mixture turned brown and muddy.

(c) The solution of 43.5 mg of sennoside A in 0.42 ml of 0.25% Na_2CO_3 deuterium oxide was heated at 85° under bubbling of N_2. After 5 or 8 hr, the protons at 10 or 10′ in sennosides were observed by NMR spectra. It was proved that 36% or 50% of protons were deuterized in 5 or 8 hr respectively.

Isomerization of Sennoside C to D——About 30 mg of sennoside C was isomerized by the same method as in item (a) above. An unknown spot, which had some proper characteristic in sennosides, was detected at *Rf* 0.45 on PPC. Although we could not confirm it, it was reasonable to assume it was sennoside D.

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