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On the Contents of Therapeutically Active Components of Russian Comfrey

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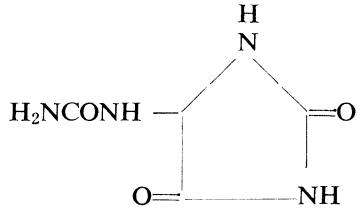
(Tables 1-3)

ELLIOT and CROFT[1] reputed that Russian comfrey was an excellent feed for cattle and cheep, because of their digestion trial of leaves, which had been artificially dried and were ground through a hammer mill before feeding. Also Russian comfrey is considered to be an ornamental plant,[2] and recently it has been studied concerning the effects of drying and storage of its root on the contents of therapeutically active components.[3)4)5)]. As the authors have already studied the nature and properties of Russian comfrey,[6)7)8)] they are in this paper to report on the contents of therapeutically active components, allantoin, tannin and mucilage.

I ALLANTOIN

Originally, allantoin had been discovered from the secreting fluid of cattle's allantois. Allantoin is said to be 5-ureidohydantoin, 5-carbamidhydantoin or glyoxyldiureide as well and it has the following structure.



Allantoin exists in the urine of mammals,[9)10)] and also in the bud of seedling. Especially, it has effects on the accelerating function of plant growth and promotes the recovering from visible injuries of plant.

Grasses are harvested frequently comparing with the usual crops as their nature and properties, but their growth is very immediate. Such a phenomenon is called the regeneration of plant.[11)]

When solar radiation and temperature are optimum, Russian comfrey has a good harvest,[12)] for the strong power of regeneration of its root which contains allantoin.[5)13)]

It can also be cut every three or four weeks from April or May to November or December. In our study the content of allantoin in all parts of Russian comfrey was determined and its physiological properties were investigated.

The content of allantoin in Russian comfrey was determined by KACZMAREK and WALICKA's method[13] which had been modified in our preliminary experiment of the concentration and volume of alkali solution, and an hour of hydrolysis.

One g of the pulverized materials is macerated with 100 ml distilled water for 2 hours at room temperature. The extract is filtered and filtrate 25 ml is hydrolyzed with 5 ml of 5 % NaOH solution for 20 minutes with condenser. If necessary, discharge with activated charcoal. Then fill up to 50 ml and glyoxylic acid in 2.5 ml of extract is determined colorimetrically as a red oxidized complex of phenylhydrazine.

Glyoxylic acid was determined by KRANER's method.[14] Prepare calibration curves in the following manner: In a 15 ml test tube place 2.5 ml of glyoxylate 10^{-3}M solution and 2.5 ml of phenylhydrazine hydrochloride solution which is prepared fresh daily. Incubate the resultant solution in a 110°C oven for about 5 minutes. Allow the solution to cool to room temperature. Add 2.5 ml each of concentrated hydrochloric acid and 1% potassium ferricynide stock solution, mix well, and allow to stand 2 minutes. Transfer to a spectrophotometer cell and obtain absorbance at 520 mμ, by means of a reference consisting of the test reagents with distilled water in place of the glyoxylate solution. Glyoxylate concentrations in unknowns are determined with reference to a calibration curve.

The result of determination of allantoin in Russian comfrey by the above-mentioned method is as follows.

Table 1. Content of allantoin in Russian comfrey

Parts of plant	Content of allantoin (mg/100g)
root (spring)	624-780
root (summer)	352-440
leaf (spring)	120-150
stem (spring)	280-350
flower (spring)	350-440

The content of allantoin is the most in spring-root, next in summer-root and flower, and the least in leaf.

KACZMAREK[13] reported that the content of allantoin of Russian comfrey was 0.743% in spring root, 0.669% in summer and 1.365% in autumn. Though Russian comfrey is a plant[16] with a high level of allantoin, KACZMAREK's result was much more than ours. It follows from this that the content of allantoin is varied in the vegetation peried.[15] KACZMAREK[3] made it public that when the root of

Russian comfrey was stored at moderate temperature (40–50°C) in a dry, well ventilated area, the material obtained was light in color and had a maximum content of allantoin and also tannin and mucilage.

Not only Russian comfrey, but many kinds of plants contain allantoin.[16)17)] ENGELBRECHT[18)] reported *Phaseolus vulgaris* contained considerable amount of allantoin in roots, hypocotyls, petioles, cotyledons and leaves, and the root was preferred for the formation or the accumulation of allantoin. KULAEVA[19)] found allantoin in the roots of 26–28 days old pumpkin plants which contained 18 amino acids. In maple sap allantoic acid and allantoin were found by WALFFGANG and other.[20)] KOYAMA[21)] reported the fresh *Ehretia thyrsiflora* Nakai bark contained 0.25–0.50% allantoin and 5.3% sucrose, and the root bark 0.53–0.86%. Allantoin is contained not only in higher plants but also in 25 species of mosses, in 8 species of liverworts, and in 9 species of sphagnum, respectively.[22)]

It is suitable nitrogen source for the growth of plant.[23)] But it is not utilized perceptibly by peas,[24)] and is found in the chlorotic shoots of *Cynodon Dactylon* pers. and when applied to normal plants it produces chlorosis.[25)]

REINBOTHE[26)27)] reports that in Russian comfrey, glyoxylic acid and CO_2 enter ureide synthesis, probably through the formation of glycine and serine. Glycine is incorporated into C atoms 2, 4, 5, and 8 of allantion. Applied purines are decomposed to allantoin, while purine antagonists such as 8-azaxanthine and benzimidazole stimulant allantion synthesis to a small extent, suggesting that allantoin is formed by purine synthesis and break down.

KRUPRA[28)29)] indicated that glycine was a precursor and glyoxylic acid a hydrolysis product of allantoin. The results of his experiments on photosynthetic assimilation of $C^{14}O_2$ and the feeding of glycine-C^{14} and glyoxylate-C^{14} indicate that in wheat seedlings the root is probably an important site of allantoin synthesis. Glycine-C^{14} was found to be a better precursor of allantoin-C^{14} than glyoxylate-C^{14}. Allantoin-C^{14} was not synthesized from urea-C^{14}. CHIERICI[30)] showed that a protracted drought caused formation of tyrosine and allantoin in beet grown up to complete development.

II TANNIN

Tannin is distributed in all parts of plant; root, trunk, leaf, bark and nut extensively, and it dissolves in water. The astringency of aqueous solution of tannin is very high, and it is utilized as a chemical to tan hides. Its existence in brewed tea used daily in Japan is prominent. Though KACZMAREK[3)] has pointed out that Russian comfrey contained tannin, the amount and the nature and properties of tannin in Russian comfrey have never been studied. In this paper, the results determining the tannin content and its nature and properties are reported. The content of tannin was estimated by LÖWENTHAL's microanalysis method.[31)]

Table 2. Content of tannin in Russian comfrey

Parts of plant	Content of tannin (mg/100g)
root (spring)	0.208-0.416
root (summer)	0.519-0.831
leaf (spring)	0.249-0.416
leaf (summer)	0.104-0.133
stem (spring)	0.207-0.415
flower (spring)	0.104-0.208

The content of tannin in the root of Russian comfrey is much more in summer than in spring. On the contrary, in the leaf, it is much more in spring than in summer. And it is more in the stem than in the flower.

The nature and properties of tannin in Russian comfrey were examined by one-dimentional method of paper chromatography. Developing solvent was nBuOH: AcOH: H_2O=5:2:6 (vol.) and color reagents were 0.1% $FeCl_3$ and 1% KCN. R*f* values of spots of catechols on paper chromatogram of the extract of Russian comfrey were about 0.59, 0.67 and 0.75. Catechols corresponding to the spots on chromatogram are dl-gallocatechol, l-epicatechol and gallic acid considered from the R*f* value and color of spots. Varieties of catechols in leaves of Russian comfrey are less than in those of tea plant.

NAKABAYASHI and others[32] isolated tannin of *Spirogyra arcta* and supposed that the main component of tannin of *Spirogyra* was gallic acid.

As to the tannin in black tea, ÔSHIMA and others[33)34)35)] reported that gallocatechin was oxidized earlier than catechin and the content of free gallic acid increased by hydrolysis of a part of gallic acid ester. They also isolated l-epicatechin and l-gallocatechin from the extract of black tea. TSUJIMURA and others[36] isolated tea tannin from green tea, and recognized that this newly isolated tannin was l-gallocatechin gallate. Tannin and Fe have effect on the quality of jute fiber adversely. In tests in which jute was fertilized with $(NH_4)_2SO_4$ at rates of 0–320 lb. N/acre, the tannin content of the bark was minimum in case of fertilized with 80 lbs. N/acre.[37]

III MUCILAGE

The mucilage content of Russian comfrey was reported by KACZMAREK.[3] But its nature and properties have never been reported. When animals, especially rabbit or goat, are fed on leaves of Russian comfrey, occasionally they excrete unusual form of excretions. In this paper the nature and properties of mucilage of Russian comfrey

were examined.

Add 100ml H_2O to 1g of the pulverized leaves of Russian comfrey and extract for one hour at room temperature, or boil for 30 minutes with condenser. After that, separate the residues with centrifuge. Determine the viscosity for 10 ml or 20 ml of filtrate 25° C by OSTWALD cappilary viscosimeter.[38)]

Table 3. Viscosity

Treatment	Time (hour)	N/10 H_2SO_4 (ml)	Time of fluid drop (sec)	Relative viscosity
Not boiled	1	0.0	10.0	1.1510
		0.1	10.0	1.1622
		0.3	9.8	1.1275
		0.4	9.5	1.0928
		0.5	8.9	1.0235
	2	0.0	9.3	1.0694
		0.1	9.3	1.0694
		0.3	8.9	1.0235
		0.4	8.8	1.0123
		0.5	8.8	1.0123
	3	0.0	9.0	1.0347
		0.3	8.7	1.0000
		0.4	8.9	1.0235
		0.5	8.9	1.0235
	4	0.0	8.9	1.0224
		0.3	8.9	1.0224
		0.4	8.9	1.0224
		0.5	8.9	1.0224
	5	0.0	8.8	1.0112
Boiled	0.5	0.0	12.1	1.3915
		0.1	12.1	1.3915
		0.3	12.1	1.3951
		0.4	12.1	1.3951
		0.5	10.0	1.1510
	1.0	0.0	9.5	1.0917
		0.1	9.5	1.0917
		0.3	9.0	1.0347
		0.4	9.0	2.0347
		0.5	8.9	1.0224
	1.5	0.0	8.7	1.0000
N/10 H_2SO_4		20 ml	8.8	1.0134

As shown in Table 3, time of fluid drop and relative viscosity of the extract at room temperature decreased gradually. Application of sulfuric acid to the extract indicated the same tendency naturally.

When the extract was boiled, viscosity decreased suddenly and the value of viscosity of the extract boiling for 1.5 hours was the same as that of distilled water.

Detection of mucilage material: Add 10 times as much water to the pulverized leaves of Russian comfrey and allow to stand for 24 hours. After an hour's shaking, separate the residues through centrifuge. Add the active charcoal to filtrate, shake for one hour and filter. Evaporate for concentration to less than one fifth volume under decreasing pressure at below 40° C. Add moreover the active charcoal, shake and filter. Add 94% methanol to it, and get white precipitate.

Dissolve a fixed quantity of the white precipitate to water and develop on thin-layer chromatography with developer of ethyl acetate : isopropanol=65:35. Spray reagent is anisaldehyde-sulfuric acid. R*f* value of spot of glucose is 0.17 and its color is light blue. R*f* value of galactose is 0.18 and color is grayish green.[41)42)43)44)45)] Authors considered that white precipitate separated from thc mucilage of Russian comfrey had been almost galactose and a small amount was glucose by the color and R*f* value of spots.

Rooted leaf cuttings of Russian comfrey were found to excrete a steady flow of sugary liquid for weeks, which MOTHES and others[39)] reported, was due to punctures originally made by insects. They reported the liquid contained almost exclusively sucrose, and it was differentiated from aphis honeydew which contained other sugar and never more than 50% sucrose. In summer and when the root system becomes too large, honeydew is not secreted in such large quantities. The authors considered that MOTHES' leaf honeydew was the same material of leaf mucilage and that was not made through punctures by insects.

OSHIBUCHI and others[40)] isolated mucilage from the root of "Tororo-aoi" (*Abelmoschus manihot*, Medic) and detected its chemical components. It was concluded that mucilage was consisted of a large amount of rhamnose and galacturonic acid, and then of a small quantity of galactose and xylose, and no other carbohydrates. From the quantitative analyses of sugar components, it has been made clear that the mucilage has only two components, rhamnose and garacturonic acid, and the molecular ratio between these components was 1 : 2. The nature and properties of mucilage between Russian comfrey and Tororo-aoi are more or less different according to the kind of plants.

SUMMARY

In this paper the following contents of therapeutically active components: allantoin, tannin and mucilage in Russian comfrey have been reported.

Allantoin exists in the urine of mammals, and also in the bud of seedling. Especially, it has effects on the accelerating function of plant growth and promotes the

recovering from visible injuries of plant. The content of allantoin is most in the spring root, next in the summer root and the flower, and least in the leaf.

Tannin is distributed in all parts of plant; root, trunk, leaf, bark and nut extensively. The amount of the tannin content in roots is much more in summer than in spring. On the contrary, in leaves it is much more in spring than in summer. And it is more in stems than in flower. Considered from the R*f* value and color of spots, catechols corresponding to the spots on paper chromatogram are dl-gallocatechol, l-epicatechol and gallic acid.

Mucilage in Russian comfrey is detected to be almost galactose and a small amount of glucose due to the color and R*f* value of spots on thin-layer chromatogram.

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ヒレハリソウの二三の特殊成分

池田　実・坪田順一

ヒレハリソウは飼料作物としてのみならず，その花は観賞用として栽培されている．最近その特殊成分の研究が行われているので，著者等はアラントイン，タンニン，粘質物について検討した．

アラントインは哺乳動物の尿や植物体中に存在し，特に植物の外傷治癒再生を助長促進する．ヒレハリソウのアラントインは夏季の根より春季の根に多く，地上部では花に多く葉に少なかった．

タンニンは植物のあらゆる部分に存在し，鞣剤に用いられ，われわれの常用する茶葉などに多い．ヒレハリソウのタンニンは春季の根より夏季の根に多く，葉では夏季より春季に多く，花には少なかった．ペーパークロマトグラフィーによるヒレハリソウのタンニンは，dl-gallocatechol, l-epicatechol, gallicacid であった．

粘質物は植物の特殊成分の一つである．ヒレハリソウの葉の水抽出物について粘度を測定した．ヒレハリソウの葉より分離した粘質物は，薄層クロマトグラフィーによる Rf の値とスポットの色よりみて，大部分は粘質物の一般成分である galactose で，一部少量の glucose が含まれていることを同定し得た．