# VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

## PART IV.—THE ACTIVE PRINCIPLES OF RHUBARB

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## INTRODUCTION

NUMEROUS attempts have been made to locate and isolate the active principles of rhubarb. Among the earlier workers in this field was Tschirch, who in his numerous reports[1,2,3,4,5,6] states that the purgative action of rhubarb depends on the oxymethylanthraquinones it contains, the free anthraquinones being less active than those combined in the form of anthraglycosides. This statement was the basis of many colorimetric and gravimetric assay processes developed in the hope that the purgative activity of rhubarb might thus be determined.

Not until 1911, when Tutin and Clewer[7] reported, as a result of their investigation, that both the free anthraquinone compounds and the crystalline anthraglycosides have very little activity, but that the non-glycosidal resin is highly active, was Tschirch's theory of active anthraglycosides doubted; also the latter's statement that there is no resin present in rhubarb.

In 1915, Wasicky[8] found that at the beginning of spring, rhubarb root contains no oxymethylanthraquinones, and yet it then acts as an efficient purgative. In 1924, in conjunction with Heinz[9], he showed that this activity was due to the presence of anthranols, from which the anthraquinones are derived. This was confirmed in 1925 by Tukats[10], who found that rhubarb gathered in winter contains anthranols, including chrysophanthranol. In 1923, Casparis and Göldlin[11] also came to the conclusion that reduced products of anthraquinones are present in rhubarb, because passing air through an alkaline solution caused a marked increase of anthraquinones. They also showed that when the active principles were subjected to high temperatures a great loss in activity resulted.

Kroeber[12] in 1923 demonstrated that when rhubarb powder was freed from oxymethylanthraquinones, using dilute sulphuric acid and chloroform, it still acted as a satisfactory laxative with 4 persons out of the 5 on whom it was tried. He concluded that the percentage content of oxymethylanthraquinones is not a reliable indication of the purgative activity of the drug. Fühner[13] in 1925 tested chrysophanol and Gilson's "rheopurgarin" on mice, and found that they were less active than the powdered rhubarb itself. He, however, showed that the active constituents were soluble in ether and methanol as when he extracted the

powdered drug with these solvents, the exhausted marc had no purgative action on mice.

In recent years, Siegrist[14] in 1932 confirmed the result of Tutin and Clewer that the crystalline anthraglycosides they isolated have very little purgative activity, and is of the opinion that there are present in rhubarb other active principles besides the anthraquinones, to which it is quite possible the main action of the drug is due.

More recently, Ström and Kihlström[15], who studied the anthraquinone contents of Chinese and rhapontic rhubarbs, in 1948 came to a similar conclusion when they stated that the chemical assay (of the anthraquinones) does not give a true picture of the purgative activity.

In Part I of this present series of papers[16], it was shown that the main activity of the anthracene derivatives of purgative drugs is displayed when they are present as glycosides of the anthranols or similar reduced forms. This generalisation probably explains most of the facts summarised above. Certain crystallisable glycosides, however, are reported to be inactive. This may be because their aglycones are anthraquinones (not anthranols); on the other hand, it may be because those components grouped as "combined anthracene derivatives"* vary considerably in activity. It was decided, therefore, to examine the combined anthracene derivatives of rhubarb in order to determine which part of this fraction is responsible for the purgative activity. For this purpose it has been necessary to devise a suitable method of chemical analysis which would give a comparatively detailed picture of the quantities of the various anthracene constituents. Furthermore, since rhubarb also contains tannin which has an antagonistic action to that of the anthracene derivatives, it has been necessary to determine the tannin content of all the samples examined. The results of these chemical assays have been compared with those of biological assays carried out in parallel. As a consequence we conclude that there is strong evidence to suggest that the combined rhein-like compounds present in rhubarb are responsible for the main purgative activity.

## Chemical Analysis of the Anthracene Derivatives

A careful consideration of the published methods for the assay of the anthracene derivatives occurring in rhubarb suggested that the most satisfactory method was the colorimetric one based on the well-known Bornträger reaction. The powdered drug is first hydrolysed and the liberated hydroxy-anthraquinones extracted with an immiscible solvent, such as ether or chloroform, and this is in turn extracted with a dilute solution of caustic alkali; the intensity of the red colour developed in the alkaline solution is then compared with that of a standard preparation.

However, none of the colorimetric methods so far described seems

* For convenience, those anthracene derivatives which occur as glycosides or resins are classified as "combined" so as to distinguish them from those occurring in the free form.

to have considered all the factors involved, e.g., whether hydrolysis of the combined anthracene derivatives is complete under the conditions prescribed. The standards used are unsatisfactory as no details of their preparation or purity are given, neither were absorption curves for the rhubarb fractions made in order to find whether the recommended standard and filter were suitable. We decided therefore to investigate the process thoroughly, dealing with each factor involved separately, and to prepare absorption curves, etc., in order to devise a suitable standard and filter. Furthermore, since some of the anthracene constituents are related to rhein, which has a carboxylic group, it was thought that by using sodium bicarbonate solution at particular stages it would be possible to estimate the total anthracene compounds under the following 4 heads: free and combined rhein-like compounds, free and combined non-rhein compounds. It was hoped that the increased information yielded by these detailed results would throw light on the relationship between the constituents and the physiological activity.

As a result of this investigation it was found necessary to observe the following conditions in devising a suitable assay process:—

(1) *Separation of the Free from Combined Anthracene Derivatives.*

This can best be accomplished by partition between an aqueous phase at about *p*H 3·0 in which the combined anthraquinones are soluble and an immiscible solvent in which the free compounds are soluble.

(*a*) *Infusion or Suspension.* One can effect this separation for senna preparations by using an infusion of the crude drug. This method, however, cannot be applied to rhubarb which, unlike senna, contains a large proportion of free compounds which are not very soluble in water, and so would not be completely extracted in an infusion. Moreover, our experiments showed that boiling water led to a slight hydrolysis of the combined anthraquinones. It was decided therefore to use a suspension of finely powdered rhubarb in cold water.

(*b*) *The Choice of Organic Solvent.* Ether is commonly used as a solvent and is specially recommended by Tschirch[5,17] and Kussmaul and Becker[9,18]. However, Fairbairn[19] suggested that ether dissolves out interfering substances from rhubarb and recommended the use of carbon tetrachloride or benzene as a solvent for the qualitative test*. Our experiments confirmed that when ether is used as a solvent the final alkaline solution is sometimes distinctly brownish, especially when the anthraquinone content of the sample is low. Carbon tetrachloride and benzene, however, have a very low solvent action on the anthraquinones, so we decided to try chloroform, which is a better solvent. The following

* Daels[20,21], Brandt[22], and Björling and Ehrlén[23] recommended the use of a 10 per cent. solution of sodium bisulphite to remove the interfering substance. This method was tried and the absorption curve of the red alkaline liquid was compared with that derived from the untreated ether extract, but no significant difference was observed. Owing to the presence of rhein compounds in the ethereal extract, solutions of ammonium carbonate and of sodium bicarbonate as recommended by Brandt, and Björling and Ehrlén are also not suitable as purifying agents.

experiment showed that ether does dissolve an interfering substance which is not dissolved by chloroform; accordingly it was decided to use chloroform in the initial extraction stages.

An aqueous suspension of the powdered drug was extracted with ether, the ether extract was exhausted with sodium hydroxide solution and this was acidified and exhausted with chloroform. When this chloroform extract was shaken out with N sodium hydroxide, a good red colour was obtained in the alkaline solution and it remained red on mild oxidation with 3 per cent. hydrogen peroxide. The acidified solution which had been extracted with chloroform was now exhausted with ether and the yellow ether solution shaken out with N sodium hydroxide. A brownish colour resulted which when mildly oxidised as above became pale yellow indicating the absence of anthraquinones.

(2) *Hydrolysis of the Combined Anthracene Derivatives.*

Experiments showed that heating in 3N acid in a boiling water-bath for 15 minutes, as recommended by Kussmaul and Becker[18] for senna, also gave the best results with rhubarb.

(3) *Oxidation of the Liberated Aglycones.*

Some of the aglycones occur in the reduced form (anthranol) and require oxidation to the corresponding anthraquinone form in order to give a red colour with sodium hydroxide solution. Kussmaul and Becker tried several oxidising agents on senna aglycones, and concluded that heating with a low concentration of hydrogen peroxide gave the best results. The rhubarb aglycones were found to be more readily oxidised than those of senna, so that a lower concentration, viz., 0·1 ml. of a 3 per cent. solution of hydrogen peroxide per 10 ml. of alkaline solution, when heated in a boiling water-bath for 4 minutes, was sufficient.

(4) *Choice of Standard and Filters.*

The four groups of anthracene derivatives were separated as follows. Suitable quantities of powdered rhubarb were suspended in cold water at *p*H 3·0 and exhausted with chloroform. This chloroform solution represents the free compounds and by means of sodium bicarbonate solution was separated into the rhein-like (i.e., those with a carboxylic group) and non-rhein compounds. The aqueous suspension which had been exhausted with chloroform was hydrolysed and the liberated aglycones were extracted with chloroform and once more separated into the rhein-like and non-rhein compounds representing the combined anthracene derivatives. The four fractions were transferred to N sodium hydroxide and oxidised as described before. The extinctions of the four resulting red-coloured solutions were read at wave-lengths between 400 and 600 mμ on the UVISPEK photoelectric spectro-photometer H700, and from the values obtained the absorption curves for each were drawn. A consideration of these curves led to the following decisions:

*(a) Free and Combined Rhein-like Compounds.*—Peak of the curve (absorption maximum) is at 500 mμ, which is exactly that of pure rhein.

Therefore it was decided to use rhein as a standard and measure the colour-intensity at a wavelength of 500 mμ.

(*b*) *Free Non-rhein Compounds.*—Maximum absorption at 505 mμ. These non-rhein compounds may consist of aloe-emodin (max.= 500 mμ)[16], emodin (max.=530 mμ)[20,21,22], chrysophanol (max.= 500 mμ)[16] and emodin monomethylether. Although Tutin and Clewer[7] found that emodin was present in the highest proportion, we found the absorption maximum of the mixture was 505 mμ, i.e., nearer aloe-emodin and chrysophanol. Chrysophanol is present in larger proportion than aloe-emodin, therefore it was decided to use chrysophanol as standard. With all these anthraquinone compounds mentioned the extinction coefficients and the molecular weights are similar, so that no great error is involved in using chrysophanol as a standard for this mixture.

(*c*) *Combined Non-Rhein Compounds.*—Maximum absorption at 500 mμ. Chrysophanol was used as a standard.

*Note.*—Since the absorption maxima of the four rhubarb fractions are practically the same, it was decided to determine the free and total anthracene derivatives and calculate the figures for the combined anthracene derivatives by deduction. In this way, not only the determinations of the free and total can be carried out simultaneously, but also the possibility of a slight loss of the combined anthracene derivatives is avoided; this loss usually occurs in the determination of the combined when the suspension of powdered rhubarb, which has been extracted with chloroform to remove the free anthracene derivatives, is transferred from the separators to the flask for hydrolysis.

## The Assay Process

1. *Free Anthracene Derivatives*

About 0·1 g.* of the air-dried, finely powdered sample is accurately weighed into a small beaker and mixed with 5 ml. of water and 2 drops of N sodium hydroxide. The mixture is transferred to a separator with the aid of 15 ml. of water, and brought to about *p*H 3 by adding N hydrochloric acid. The acidic suspension is then exhaustively extracted by shaking with successive quantities of chloroform until the chloroform layer becomes colourless. The chloroform extracts are combined, washed with a small quantity of water acidified with hydrochloric acid, and filtered. The clear filtrate is exhausted with 5 per cent. sodium bicarbonate solution and the bicarbonate extracts are combined and washed with a small quantity of chloroform and the washings added to the main chloroform fraction containing the non-rhein compounds.

(*a*) *Rhein-like Compounds.* The combined bicarbonate fraction is

* This quantity has been selected after considerable experience and careful consideration. If smaller quantities are used, the results will be less accurate due to greater sampling error; if larger quantities are used, the mucilage present in the drug will cause persistent emulsion during extraction with chloroform and also inconveniently larger quantities of chloroform will be needed in order to extract the free anthracene derivatives completely.

acidified by adding dilute sulphuric acid and exhausted with ether. The ethereal extracts are combined and, after washing with a small quantity of water acidified with hydrochloric acid, completely extracted with N sodium hydroxide. The combined alkaline solution containing only the rhein-like compounds is then heated in a vigorously boiling water-bath for 4 minutes, cooled under the tap, and diluted to such a volume that a suitable colour intensity reading may be obtained when measured in a colorimeter; as the coloured solution is not very stable, its colour intensity should be read within 30 minutes.

(*b*) *Non-rhein Compounds.* The chloroform solution which has been extracted with sodium bicarbonate solution is completely extracted with N sodium hydroxide, and the alkaline solutions treated as above and the intensity of the colour measured. The intensity of the colour solutions were measured in an electrophotometer, viz., the EEL test tube colorimeter, with filter No. 623 (green) which transmits light of wave-lengths between 470 mμ and 530 mμ with maximum transmission at about 495 mμ. The concentration of the rhein-like compounds in the colour solution was calculated from a calibration curve for pure rhein and that of the non-rhein compounds, from a calibration curve for pure chrysophanol.

2. *Total Anthracene Derivatives*

About 0·1 g. of the air-dried, finely powdered sample is accurately weighed into a small beaker and mixed with 5 ml. of water and 2 drops of N sodium hydroxide. The mixture is transferred to a 250-ml. flask with the aid of 15 ml. of water. After adding 10 ml. of 10 N sulphuric acid the mixture is heated in a boiling water-bath under a reflux condenser for 15 minutes. 100 ml. of chloroform is then added slowly from the top of the condenser and the heating continued for another 5 minutes. The contents of the flask are then thoroughly cooled under the tap and transferred to a separator. The chloroform layer is separated, washed with 5 ml. of water acidified with hydrochloric acid and filtered. The aqueous layer is completely extracted with further quantities of chloroform. The chloroform extracts are washed, filtered and combined. The combined chloroform solution is then exhausted by shaking with 5 per cent. sodium bicarbonate solution to separate the rhein-like from the non-rhein compounds. Each of these two fractions is transferred into N sodium hydroxide, and since they may contain reduced aglycones, they are oxidised by heating with 0·1 ml. of 3 per cent. hydrogen peroxide per 10 ml. of alkaline solution in a boiling water-bath for 4 minutes and their colour-intensities measured as described for the corresponding free compounds. The values for the combined compounds may be obtained by deducting the values for the free compounds from those for the total compounds.

## Assay of Tannins

Soos[26] has made a thorough review of the methods used for the assay of tannins, and concludes that those based on the agglutination of red

blood cells give the best indication of the astringent action. He describes a method, based on that of Kobert[27] and Wasicky[28] involving the use of red blood cells, and it was decided to use this method on rhubarb as it is the astringent action of the tannins present which effects the purgative action of the anthracene derivatives.

1. *Preparation of the Rhubarb Extract.* A 4 to 5 per cent. extract of rhubarb was made as follows: 2·0 to 2·5 g. of the powder was weighed into a 100-ml. flask, mixed with about 45 ml. of boiling distilled water and kept in a boiling water-bath under a reflux condenser for 15 minutes. After allowing to stand at room temperature for 1 hour it was cooled under the tap. The contents were transferred to a measuring flask, rendered isotonic to blood with sodium chloride and made up to 50 ml. with cold distilled water. The mixture was then centrifuged. The supernatant liquid was used for the test and was found to be quite stable for a week when kept in a refrigerator.

2. *The Test.* 0·5, 1·0, 2·5 and 5·0 ml. of the rhubarb extract were pipetted into 4 test tubes respectively and each made up to 5 ml. with physiological saline solution. 2·5 ml. of a 2 per cent. blood cell suspension* was added to each of these tubes and these were shaken to mix the contents and allowed to stand at room temperature for about 24 hours. By that time, some of the tubes showed complete agglutination of the blood cells at the bottom and the supernatant liquid became yellow or brownish, while the others showed very little agglutination and the supernatant liquid still remained reddish in colour. A portion of the supernatant liquid was pipetted into a clean test-tube and a few drops of 0.2 per cent. ferric chloride solution were added to see whether all the tannins originally present had been used up for the agglutination of the blood cells. The lowest concentration of the rhubarb extract which caused 100 per cent. agglutination of the blood cells was noted and the test was repeated using a further suitable dilution of the rhubarb extract so that a more accurate determination of the end-point could be obtained.

At the same time similar experiments were carried out using a standard tannin solution (0.2 per cent. of tannic acid, B.P., in physiological saline solution) in the same way as that described for the rhubarb extract. The astringent value of the standard tannic acid was taken as 100 and that of the sample of rhubarb calculated accordingly.

## Comparison of Results of Biological and Physico-Chemical Assays

The following samples of rhubarb were assayed for their purgative activity (by the method of Lou[29]), astringent value of the tannins present, and the content of anthracene derivatives. The results of these assays are recorded in Table I.

Sample 1. Chinese Rhubarb—purchased from a wholesaler, London, 1949; used as the Laboratory standard ($R_S$).

* Soos[26] used horse blood, but human blood was used in our experiments.

## TABLE I

RESULTS OF BIOLOGICAL AND PHYSICO-CHEMICAL ASSAYS OF RHUBARB

| Samples of Rhubarb | Biological Assay ($R_S$=100) | Chemical Assay, in percentages | | | | | | | | | Tannin Assay (Astringent Value) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FREE | | | COMBINED | | | TOTAL | | | |
| | | Rhein | Non-rhein | Total | Rhein | Non-rhein | Total | Rhein | Non-rhein | Total | |
| Chinese | | | | | | | | | | | |
| (1) $R_S$ ... ... | 100 | 0·42 | 0·88 | 1·30 | 0·64 | 1·24 | 1·88 | 1·06 | 2·12 | 3·18 | 5·7 |
| (2) ... ... | not assayed | 0·31 | 0·86 | 1·17 | 0·76 | 1·24 | 2·00 | 1·07 | 2·10 | 3·17 | 5·0 |
| (3) ... ... | „ „ | 0·44 | 0·81 | 1·25 | 0·53 | 1·17 | 1·70 | 0·97 | 1·98 | 2·95 | 5·0 |
| English rhapontic | | | | | | | | | | | |
| (4) ... ... | 58 | 0·13 | 0·62 | 0·75 | 0·41 | 1·33 | 1·74 | 0·54 | 1·95 | 2·49 | 3·3 |
| (5) ... ... | 44 | 0·08 | 0·57 | 0·65 | 0·37 | 0·99 | 1·36 | 0·45 | 1·56 | 2·01 | 5·0 |
| (6) ... ... | not assayed | 0·16 | 0·67 | 0·83 | 0·21 | 0·85 | 1·06 | 0·37 | 1·52 | 1·89 | 5·0 |
| French rhapontic | | | | | | | | | | | |
| (7) ... ... | very low | traces | 1·49 | 1·49 | traces | 1·25 | 1·25 | traces | 2·74 | 2·74 | 2·0 |
| Austrian rhapontic | | | | | | | | | | | |
| (8) ... ... | very low | traces | 1·42 | 1·42 | traces | 0·98 | 0·98 | traces | 2·40 | 2·40 | 3·3 |

Sample 2. Chinese Rhubarb—obtained from a wholesaler, London, 1950.

Sample 3. Chinese Rhubarb—obtained from a wholesaler, London, 1950.

Sample 4. English Rhapontic Rhubarb—purchased from a wholesaler, London, 1942.

Sample 5. English Rhapontic Rhubarb—obtained from a wholesaler, London, 1950.

Sample 6. English Rhapontic Rhubarb—collected from a drug farm at Hitchin, Hertfordshire, 1949, sliced and dried in the laboratory.

Sample 7. French Rhapontic Rhubarb—obtained from a wholesaler, London, 1950.

Sample 8. Austrian Rhapontic Rhubarb—obtained from a wholesaler, London, 1950.

A careful examination of the results recorded in Table I shows that:

(1) The content of total anthracene derivatives gives no indication of the purgative activity. The English, French and Austrian rhubarbs contain similar quantities of these compounds, yet their purgative activities differ to a considerable extent.

(2) The content of free anthracene derivatives gives no indication of the purgative activity. Samples 7 and 8 contain the highest proportions of these free compounds, yet they have a very low purgative action.

(3) The content of total combined anthracene derivatives does not give a clear indication of the purgative activity, as can be seen by comparing Samples 4, 5, 7 and 8 with Sample 1.

(4) The content of non-rhein compounds either in the free or in the combined state gives no indication of the purgative activity. Samples 7 and 8 contain the highest proportions of the non-rhein compounds, but they have very low purgative action.

(5) The purgative activity runs parallel with the content of *rhein-like* compounds. Since pure rhein has no purgative effect (Fairbairn[16]), it is logical to conclude that the combined rhein-like compounds may be responsible for the purgative action of rhubarb. This can be seen from Table I, where the results of the biological assays, shown in heavy black type, show a much closer correlation with those figures, also in heavy black type, in the column " combined rhein " than those in any other column (except for " total rhein ").

The results of the tannin assays do not affect this conclusion, since Sample 1, which has the highest purgative activity, has also the highest tannin content; samples 7 and 8 with a very low " combined rhein " content also have a comparatively low tannin content, so that their low purgative activity cannot be accounted for by the presence of an abnormally large amount of tannin.

As further evidence for the conclusion in paragraph (5) above, a

sample of Chinese rhubarb (Sample 1,$R_S$) was extracted in a Soxhlet apparatus for 2 days using ether, then methylal as solvent, and the marc dried ($R_{ex}$). Chemical analysis showed that most of the free compounds and also the "non-rhein" compounds had been removed; but only about one-third of the "combined rhein" and one-third of the tannins had been removed. Biological assay showed that approximately one-third of the purgative activity had also been lost. These results are shown in Table II.

TABLE II

RESULTS OF BIOLOGICAL AND PHYSICO-CHEMICAL ASSAYS ON CHINESE RHUBARB ($R_S$) AND EXHAUSTED RHUBARB ($R_{ex}$)

| | Biological Assay | Chemical Assay | | | Tannin Assay (Astringent Value) |
|---|---|---|---|---|---|
| | | Combined Rhein | Combined non-Rhein | Free Compounds | |
| | | per cent. | per cent. | per cent. | |
| $R_S$ ... ... ... ... | 100 | 0·64 | 1·24 | 1·30 | 5·7 |
| $R_{ex}$ ... ... ... ... | 63 | 0·40 | 0·15 | 0·25 | 4·0 |

## DISCUSSION

Recently, Mühlemann[30] has synthesised several anthraquinones and and their glycosides and determined their relative purgative activities by the method of Fühner[13] and Uhlmann[31]. The results showed that chrysophanol glucoside was four times as active as chrysophanol and that emodin glucoside had the same activity as emodin. The minimum effective dose of the chrysophanol and emodin glucosides was about 4 mg./20 g. of mouse (i.e., 0·2 mg./g. of mouse).

If the assumption is made that the non-rhein compounds of rhubarb consist largely of chrysophanol and emodin, free and combined in the form of glucosides, the results recorded in Table I can be compared with the figures given by Mühlemann. To make the comparison easier it can be further assumed that all of these compounds have the same activity, viz., a minimum effective dose of 4 mg./20 g. mouse. The minimum effective dose of Chinese rhubarb as given by Fühner[13] is 5 to 10 mg./20 g. of mouse and this accords with our experience with Sample $R_S$, the minimum effective dose of which is about 8 mg. This dose contains about 0·17 mg. of chrysophanol and emodin (free and combined); hence this quantity can only represent 0·17/4·0 or 4·3 per cent. of the activity of the rhubarb. While several assumptions have been made in this calculation, they would not materially affect the order of magnitude of the figure arrived at. This figure confirms the conclusion which we have reached, viz., that the non-rhein compounds of rhubarb contribute very little to the purgative activity.

The evidence therefore points very strongly to the fact that the purgative activity of rhubarb is due to the combined rhein fraction. This statement, however, cannot be regarded as an established fact until the components of this fraction have been isolated in a pure state and their

activity compared directly with that of a corresponding amount of rhubarb. This work is now in progress in this laboratory.

It is also interesting to point out at this stage the similarity which exists between the purgative principles of senna, sennosides A and B the aglycones of which have a carboxyl group[32], and the rhein-like compounds of rhubarb which also have a carboxyl group.

## Summary

1. A study of those anthracene derivatives of rhubarb which occur in the glycosidal (or perhaps more accurately, in the "combined") form has been made.

2. For this purpose a method of chemical assay has been devised which enables the amount of anthracene derivatives present to be determined under the following four headings: free and combined rhein-like compounds; free and combined non-rhein compounds.

3. Several samples of powdered rhubarb have been analysed by this method and the results compared with the biological activity (by the method of Lou[29]) and tannin content (by the method of Soos[26]) of the samples (see Table I).

4. The above comparison revealed that the biological activity runs parallel with the content of combined rhein-like compounds; free rhein has practically no purgative activity[16].

5. This was further proved by removing practically all the anthracene derivatives from a sample of rhubarb, except for about 2/3 of the combined rhein-like compounds. Table II shows that the exhausted rhubarb still retained 2/3 of the original activity.

6. Further work is proceeding on the isolation of this active material.

7. English rhapontic rhubarb contains less of the active rhein-like compound than Chinese rhubarb, and samples of Austrian and French rhapontic rhubarb contained only traces.

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