# VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

## PART VII. THE EVALUATION OF CASCARA SAGRADA AND ITS PREPARATIONS

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### INTRODUCTION

CASCARA bark is the dried bark of *Rhamnus purshianus*, D.C. collected at least one year before being used. It has been used as a tonic laxative in medicine since about 1804, and is now a well-established drug for the treatment of constipation. However, there is still no generally accepted method of measuring the activity apart from determining the water-soluble extractive, which is obviously a rather crude method of assessing activity. Numerous methods of chemical assay have been published based on the determination either by gravimetric or colorimetric means of the amount of anthraquinone compounds present, but no-one has been able to correlate their results with the biological activity. Fairbairn[1] has already shown the importance of the form in which the anthraquinones occur and has demonstrated that for cascara, the free anthraquinones contribute little to the purgative activity. He has also shown that for senna and rhubarb, the main activity is associated with the glycosidal or "combined" anthracene derivatives. We therefore decided to estimate the amount of anthracene glycosides in various samples of cascara bark and its preparations and see if the results were correlated with the biological activity.

### DETERMINATION OF THE CONTENT OF ANTHRACENE GLYCOSIDES

As already stated, numerous methods of chemical assay of cascara and the closely related drug frangula (*Rhamnus frangula* L.) have been published. Most of them are based on the red colour produced by the well-known Bornträger reaction or one of its modifications; the intensity of the colour is matched against standard solutions of anthraquinones in alkali, or measured photoelectrically. It is obvious, however, that a colorimetric method will only be satisfactory if the anthraquinones can be extracted in a fairly pure form for measuring, but few of the published methods have taken into account the fact that interfering substances are present in cascara and readily pass into the final solution being measured. Furthermore, the absorption curves of the final red colour are seldom given so that it is not possible to know whether the standard used or the wave-length are suitable. In view of our experience with the chemical assay of senna[2] and rhubarb[3], we decided to apply similar methods of colorimetric assay to cascara. This necessitated carefully testing each stage of the process, e.g., removal of free compounds, hydrolysis, etc., as

they applied to cascara. As a result, we found difficulties peculiar to cascara and they had to be overcome before a satisfactory method was evolved.

*Difficulties Peculiar to the Chemical Assay of Cascara.* Cascara contains a much larger proportion of free anthraquinones than does senna, so that the removal of these requires a comparatively large amount of organic solvent. If ether is used for this purpose as in the senna assay[2], a certain amount of glycoside is also removed and cannot be recovered conveniently. Consequently we used chloroform instead, as had been used with rhubarb.[3] A more serious difficulty was the presence of some pigments which were carried with the liberated aglycones into the final alkaline solution and imparted to it a dirty brown tint. This interfering substance has already been reported by Fairbairn[4] and several other workers have attempted to destroy it. Thus Brandt[5] proposed a 5 per cent. solution of totally effloresced ammonium carbonate. Björling and Ehrlén[6] working on frangula used sodium metabisulphite and sodium bicarbonate and Fairbairn and Lou[3] found that the use of potassium metabisulphite was sufficient for the removal of interfering substances in the assay of rhubarb. Gibson and Schwarting[7] used a chromatographic method of assay and claimed that they could obtain the anthraquinones in a pure form and separate from a yellow pigment which was retained on the column.

We decided to test these methods for eliminating the interfering substance by preparing the absorption curves of the final coloured solutions and comparing them with those of pure aloe-emodin and of pure emodin in alkaline solution. Glycosides of aloe-emodin[8] and of emodin[9] have already been shown to occur in cascara. In view of the fact that Gibson and Schwarting publish absorption curves of their purified fractions and claim they are identical with those of the pure anthraquinones, we firstly tried out their chromatographic method. A disadvantage common to most methods of absorption chromatography is the difficulty of obtaining consistency in successive batches of absorbent; this difficulty is increased when, as in Gibson and Schwarting's work, a commercial product like "Celite" was used. However, we managed partly to overcome this difficulty by obtaining from America supplies of celite and the special magnesia used. Another serious objection to the method was the fact that it took the authors over a week to develop the column; we found that even after 10 days' elution, no differentiation of the anthraquinones had occurred. It is obvious that even if we had been able to differentiate and elute all the anthraquinones, the method would still be inconvenient in practice. We did make further attempts at using absorption chromatography with sucrose, magnesium carbonate, magnesium oxide, alumina and charcoal as absorbents, but were unsuccessful and we concluded that while absorption chromatography is extremely useful for qualitative work, it was not so useful for quantitative work.

We made further attempts at eliminating the interfering substances by the use of various organic solvents to extract the aglycones after hydrolysis and by treatment of these solutions with metabisulphites and bicarbonates

as advocated by Brandt[5], Björling and Ehrlén[6] and others, but in no instance were the interfering substances completely removed (*see* Figure 1 (b)).

During the course of the work, it was noticed that a sample of bark about 6 years old had little interfering substance in the final solution (*see*

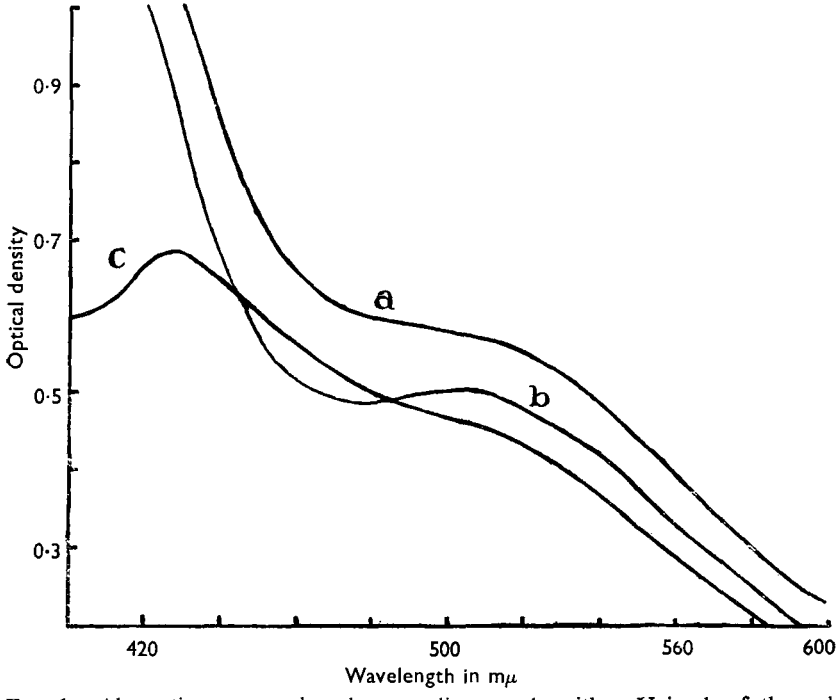


FIG. 1. Absorption curves, based on readings made with a Uvispek of the red alkaline solutions prepared from (a) 1 year old bark $C_3$ without special treatment; (b) $C_3$ after metabisulphite and bicarbonate purification stage; (c) 6 year old bark $C_1$.

Figure 1 (c)). A comparison of the curves 1 (b) and 1 (c) shows that the metabisulphite treatment has removed some colour interfering at about 480 mμ and that prolonged storage has removed substances interfering at about 420 mμ. A combination therefore of these two methods of treatment might well remove the bulk of the interfering substances. It is generally assumed that during storage, a griping or an emetic principle is gradually destroyed; furthermore some workers claim that this prolonged natural process can be substituted by treating the bark with hydrogen peroxide. Though there was no reason to connect the interfering substances with this griping principle, we decided to try whether treatment with peroxide at various stages in our chemical assay process would destroy some of the interfering substances. This quite empirical idea turned out to be successful in practice, though another equally empirically determined stage was necessary before the final solution was pure enough for colorimetric assay. We found that if a fairly large proportion of hydrogen peroxide was added during the acid hydrolysis of the glycosides, and the final

alkaline solution of the liberated aglycones was also oxidised with hydrogen peroxide as in the assay of the other anthraquinone drugs, the solution was practically free from interfering substance. If, however, the final alkaline solution was acidified, extracted with ether and the ether solution re-extracted with sodium hydroxide solution this new alkaline solution is now quite pure as is indicated by its absorption curve which is very close to that of a mixture of aloe-emodin and emodin (Figure 2).

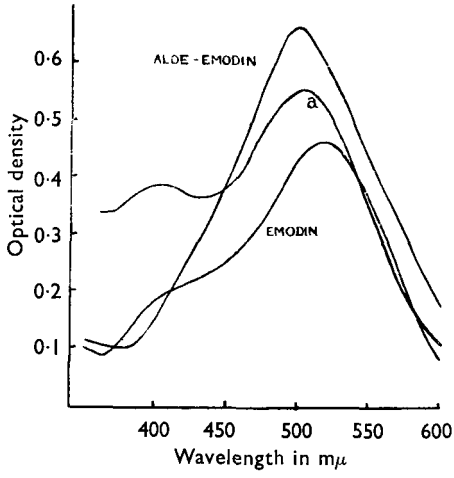


FIG. 2. Absorption curves, based on readings made with a Uvispek, of the red alkaline solutions prepared from (a) cascara by the method advocated in this paper, from pure aloe-emodin; (21·4 mg./l.; $E^{1\ per\ cent.}_{1\ cm.}$ = 308) and pure emodin, (16·8 mg./l.; $E^{1\ per\ cent.}_{1\ cm.}$ = 274).

Further experiments showed that the conditions for dissolving the glycosides and hydrolysing them and for the alkaline oxidation of the aglycones as used for senna and rhubarb were equally applicable to cascara. We also passed a known amount of pure aloe-emodin through all the stages of the assay process and found there was no loss involved.

The peak of the absorption curve of the final alkaline solution is 500 mμ; that of emodin is 520 mμ and that of aloe-emodin is 500 mμ. This suggests that aloe-emodin is present in the final alkaline solution in greater proportion than emodin, and it was therefore decided to use pure aloe-emodin as a standard and measure the intensity of the colour at 500 mμ. Since the molecular weights and extinction coefficients of both compounds are similar; there will be little error in using this standard and, as will be shown later, the results of the chemical assays, calculated in terms of aloe-emodin, are closely correlated with those of the bioassays. Details of the chemical assay process appear at the end of this paper.

The fact that the interfering substances partly disappear with age may be of use in determining the approximate age of a sample of cascara bark.

## THE BIOLOGICAL ASSAY

The biological assay was based on that of Lou and Fairbairn[10] for cascara. In contrast to their earlier method, however, we were able to use the crude drug as standard providing a fairly potent sample was used, it was in fine powder and that 1 ml. of suspension was given to each mouse.

As a standard, we used a good sample of powdered cascara bark ($C_S$) in the same way as powdered senna pod ($P_1$) and powdered rhubarb ($R_S$) had been used with the other anthraquinone drugs. However, in order to relate the activities of our cascara standard with that of other drugs and

particularly with that of a pure compound, we assayed $C_S$ against $P_1$ (Table I) and against pure sennosides A + B (two assays). It was found that

1 g. of Cascara $C_S \equiv 0.185$ g. of senna pod $P_1$
1 g. of $P_1 \equiv 58$ mg. of sennosides A + B*
1 g. of $C_S \equiv 10{\cdot}7$ mg. of sennosides A + B

*Accuracy of the Bioassay Process.* Table I shows the results of 10 replicate assays of $C_S$ against $P_1$ as standard and Table II the results of 11 replicate assays of another sample of powdered cascara $C_4$ against $C_S$ as standard. In Table I the potency of $P_1$ is taken to be 100, and the mean of the values for the potencies of $C_S$ is found to be 18·5, with a standard deviation of 1·27, i.e., 6·9 per cent. of the mean. In Table II the potency of $C_S$ is taken to be 100 and the mean of the values for the potency of $C^4$ is found to be 133 with a standard deviation of 16·4, i.e., 12·4 per cent. of the mean. The limits of error for a single determination (P = 0·99) based on these figures would be ± 17·8 per cent. of the mean and ± 31·9 per cent. of the mean; these limits compare well with those given by Lou for senna[12] and Lou and Fairbairn[13] for rhubarb. As we always carry out the bioassay in duplicate, the error of the mean based on these two determinations should very rarely exceed ± 23 per cent.

TABLE I

RESULTS OF REPLICATE BIOASSAYS OF CASCARA BARK $C_S$ AGAINST SENNA POD $P_1$ AS STANDARD

| Bioassay | Potency of $P_1$ | Potency of $C_S$ |
|---|---|---|
| 1 | 100 | 17·5 |
| 2 | 100 | 18·2 |
| 3 | 100 | 17·8 |
| 4 | 100 | 19·4 |
| 5 | 100 | 20·2 |
| 6 | 100 | 15·7 |
| 7 | 100 | 18·8 |
| 8 | 100 | 19·0 |
| 9 | 100 | 18·6 |
| 10 | 100 | 19·5 |
| Mean | 100 | 18·5 |

Standard deviation = 1·3 (6·9 per cent. of the mean)

## CORRELATION OF CHEMICAL AND BIOLOGICAL ASSAYS

7 different samples of cascara bark, purchased within the last 8 years, were assayed chemically and biologically and the results recorded in Table III show that there is a close correlation between the two sets of figures. The standard sample $C_S$ was exhausted with chloroform to remove the free compounds; there was no loss in activity, thus confirming the general statement made by Fairbairn[1], that the free compounds contribute very little to the activity of the anthracene purgatives. When a similar

TABLE II

RESULTS OF REPLICATE BIOASSAYS OF CASCARA BARK $C_4$ AGAINST CASCARA BARK $C_S$ AS STANDARD

| Bioassay | Potency of $C_S$ | Potency of $C_4$ |
|---|---|---|
| 1 | 100 | 123·1 |
| 2 | 100 | 126·0 |
| 3 | 100 | 135·0 |
| 4 | 100 | 162·2 |
| 5 | 100 | 135·8 |
| 6 | 100 | 135·5 |
| 7 | 100 | 113·7 |
| 8 | 100 | 124·8 |
| 9 | 100 | 106·8 |
| 10 | 100 | 149·1 |
| 11 | 100 | 150·0 |
| Mean | 100 | 132·9 |

Standard deviation = 16·4 (12·4 per cent. of the mean)

* This figure is in close agreement with an earlier estimation where Fairbairn and Saleh[11] found that 1 g. of $P_1 \equiv 64$ mg. of Sennosides A + B.

series of assays were carried out on various batches of dry extract o cascara B.P. a curious fact came to light. Though the chemical and biological assay results were correlated, the biological assay results were all about one half of the value to be expected from the chemical assays. Thus if a dose of crude drug containing say 10 mg. of glycoside had a biological activity of 100 units, a dose of dry extract containing 10 mg. of glycoside had only an activity of 50 units. This reduction in activity was also found to hold for samples of liquid extract, elixir and tablets.

TABLE III

CHEMICAL AND BIOLOGICAL ASSAYS OF COMMERCIAL SAMPLES OF CASCARA BARK

| Sample | Water soluble extractive | Chemical assay, mg. of glycosides per g. (as aloe-emodin) | Biological assay | | B/C ratio |
|---|---|---|---|---|---|
| | | | mg. of $P_1$ per g. | mg. Sennosides A + B per g. | |
| $C_3$—received 1951 .. | 23·77 | 11·7 | 269 (mean of 3 assays) | 16·14 | 1·38 |
| $C_4$—received 1946 .. | 24·92 | 11·9 | 242 (mean of 6 assays) | 14·52 | 1·22 |
| $C_6$—received 1948 .. | 24·12 | 11·8 | (i) 225, (ii) 259 } 245 | 14·7 | 1·25 |
| $C_7$—received 1947 .. | 23·34 | 12·0 | 252 | 15·1 | 1·26 |
| $C_8$—received 1945 .. | 23·00 | 8·6 | 170 | 10·2 | 1·18 |
| $C_{10}$—received 1953 .. | | 13·25 | 265 | 15·9 | 1·20 |
| C8—received 1952 .. | 24·49 | 9·5 | 185 (mean of 10 assays) | 11·1 | 1·168 |
| C8—exhausted with chloroform to remove the free compounds .. .. | | 9·95 | 184 (mean of 4 assays) | 11·04 | 1·11 |
| $C_1$—received 1945 .. | | 9·0 | | | |
| $C_2$—received 1945 .. | | 8·0 | | | |
| $C_5$—received 1952 .. | | 8·62 | | | |
| $C_9$—received 1952 .. | | 5·75 | | | |

We have attempted to express this fact by calculating the ratio of biological activity to glycosidal content for each sample examined; this ratio is called the B/C ratio and is obtained as follows:

$$B/C = \frac{\text{Biological activity of 1 g. expressed as mg. of sennosides A + B}}{\text{Chemical assay for glycosides per g. expressed as mg. of aloe-emodin.}}$$

We are attempting at present to find out why the B/C ratio for the crude drug is twice that for the galenical preparations.

The results, however, show that the chemical assay provides a good criterion of the biological activity provided samples of crude drug are being compared with each other, or samples of galenicals are being compared. They also give some indication of the low efficiency of the present methods of preparing galenicals of cascara sagrada. The mean glycosidal content of the 11 samples of bark in Table III is 10·0 mg./g. On the assumption that the water-soluble extractive of B.P. samples of bark is 25 per cent., dry extract of cascara should contain 40 mg. of glycosides

per g. The mean for the 7 commercial samples in Table IV is only 11·1 mg./g. thus indicating that only 28 per cent. of the original glycosidal content has been retained. Similar calculations for the commercial samples of liquid galenicals mentioned in Table IV show an average yield of only 29 per cent. In the same way calculations based on the figures given by Gibson and Schwarting[14] show a similar low efficiency of about

TABLE IV

CHEMICAL AND BIOLOGICAL ASSAYS OF COMMERCIAL SAMPLES OF GALENICAL PREPARATIONS OF CASCARA

| Sample | Water soluble extractive | Chemical assay, mg. of glycosides per g. or ml. (as aloe-emodin) | Biological assay | | B/C ratio |
|---|---|---|---|---|---|
| | | | mg. of $P_1$ per g. or ml. of extract | mg. of sennosides A + B per g. or ml. of extract | |
| Dry extract—$E_1$ .. | | 17·8 | 200 (mean of 7 assays) | 12·0 | 0·67 |
| Dry extract—$E_2$ .. | | 9·0 | 105, 126 } 115 | 6·9 | 0·76 |
| Dry extract—$E_6$ .. | 87·0 | 12·25 | 135 (mean of 3 assays) | 8·1 | 0·66 |
| Dry extract $E_9$ .. | 80·4 | 11·0 | 140, 90 } 115 | 6·9 | 0·63 |
| Dry extract—$E_3$ .. | 84 | 11·75 | | | |
| Dry extract—$E_4$ .. | 86 | 5·0 | | | |
| Dry extract—$E_5$ .. | 81 | 10·8 | | | |
| Tablet (5 gr.)—$T_1$ .. | | 4·9 (mg. per tablet) | 51 (mg. $P_1$ per tablet) | 3·0 (mg. per tablet) | 0·61 |
| Tablet (5 gr.)—$T_2$ .. | | 3·0 (mg. per tablet) | 34 (mg. $P_1$ per tablet) | 2·04 (mg. per tablet) | 0·68 |
| Tablet (5 gr.)—$T_3$ .. | | 4·04 (mg. per tablet) | 42 (mg. $P_1$ per tablet) | 2·52 (mg. per tablet) | 0·62 |
| Liquid extract—$LE_1$ | | 3·3 | 31, 40 } 35 | 2·1 | 0·64 |
| Liquid extract—$LE_2$ | | 2·9 | 35, 35 } 35 | 2·1 | 0·7 |
| Liquid extract—$LE_3$ | | 3·9 | 44 | 2·64 | 0·67 |
| Elixir—$LX_1$ .. | | 2·14 | 21 | 1·26 | 0·58 |
| Elixir—$LX_2$ .. .. | | 2·02 | 14 | 0·84 | 0·42 |
| Elixir $LX_3$ .. .. | | 3·26 | 19 | 1·14 | 0·35 |

20 to 30 per cent. Though the method of chemical assay used by these authors did not then take into account the presence of an interfering substance, their figures are probably a sufficiently good guide for comparative purposes. Since the B/C ratio for all these preparations is only half of that for the crude drug it appears that they represent only about 15 per cent. of the original biological activity. Work on improving the present methods of preparing galencials is being carried out.

## THE CHEMICAL ASSAY PROCESS

About 1·0 g. of the powdered bark, accurately weighed is transferred to a graduated 100-ml. flask by means of about 80 ml. of hot water and

the flask placed in a boiling water bath for 15 minutes. The flask is cooled to room temperature, the *p*H of the solution adjusted to 6 and the contents shaken vigorously; it is then made up to volume and filtered or centrifuged. Extracts and liquid preparations of cascara are suitably diluted, adjusted to *p*H 6 and made up to a definite volume.

(i) *Removal of the free Anthraquinones.* 10 ml. of the above filtrate or solution is brought to *p*H 3 by addition of N hydrochloric acid and shaken vigorously with successive quantities, each of 30 ml., of chloroform till the chloroform is colourless after shaking. The combined chloroform extracts are washed with 2 × 5 ml. of water and the washings added to the aqueous fraction. The chloroform layer which contains all the free compounds is now discarded.

(ii) *Hydrolysis of the glycosides.* To the aqueous fraction, which now measures about 20 ml., is added 12 ml. of 6 per cent. hydrogen peroxide and 16 ml. of 10 N sulphuric acid, thus making the final concentration of acid about 3·3N. The mixture is heated for 15 minutes in a boiling water bath and then cooled under the tap, when the aglycones may appear as a brown precipitate.

(iii) *Extraction and Purification of the Aglycones.* The previously cooled liquid is shaken vigorously in a separating funnel with 80 ml. of ether, and allowed to stand. The lower aqueous portion is separated and the yellow ethereal layer decanted from the brown residue which forms as a layer between the ether and aqueous layers. The brown layer is dissolved in a small quantity of sodium hydroxide solution and this solution is added to the separated aqueous portion (which contains an excess of acid). The extraction is continued in a similar manner with successive portions of ether, until the ether fraction is colourless. The combined ether fractions are shaken with 3 quantities, each of 20 ml., of 10 per cent. sodium metabisulphite solution and with 3 or 4 quantities, each of 20 ml., of 1 per cent. sodium bicarbonate solution, the aqueous layers being discarded.

*Colorimetric Estimation.* The purified ether solution is extracted with 10 to 20 ml. of N sodium hydroxide, in small portions, till the alkaline extract is colourless. To the combined alkaline solution is added 0·2 ml. of 3 per cent. hydrogen peroxide per 10 ml. and heated for 4 minutes in a boiling water bath. The solution is rapidly cooled, excess of sulphuric acid is added and the liquid extracted with ether. The ether solution is re-extracted with N sodium hydroxide; the alkaline solution is warmed to drive off dissolved ether and made up to suitable volume. The intensity of the red colour is determined photoelectrically at 500 m$\mu$, within half an hour, and the amount of aglycone present, calculated as aloe-emodin, is read off from calibration curves previously prepared from pure aloe-emodin.

## Summary

1. A satisfactory colorimetric method for the determination of the amount of anthracene glycosides in samples of cascara sagrada and its preparations is described.

2. The main difficulty encountered was the presence of pigments

which passed into the final coloured solution being measured; it was found possible to destroy these pigments by oxidation in acid medium and by treatment, at one stage in the assay process, with sodium metabisulphite and sodium bicarbonate.

3. The method of biological assay described by Lou and Fairbairn[13] has been used extensively and we have found that the standard error of the mean based on two assays ($P = 0{\cdot}99$) was never more than $\pm 23$ per cent.

4. 7 samples of crude drug were assayed chemically and biologically by the above methods and the results showed very good correlation; similarly with 12 commercial samples of dry extract, liquid extract and elixir.

5. One curious fact, however, came to light; galenical preparations containing glycosides in amounts similar to those in the crude drug were only half as active. This problem is being investigated further.

6. The results show that commercial samples of official cascara preparations contain only about 15 per cent. of the original activity of the crude drug; we are attempting to devise more efficient methods of preparation.

We wish to thank Professor H. W. Youngken, Jnr., University of Washington, Seattle, for supplying us with the celite and the special magnesia used in the chromatographic work; Mr. J. H. Davey for assistance with the biological assays; Dr. A. L. Glenn, Department of Chemistry, School of Pharmacy, for advice in using the Uvispek spectrophotometer in his department in order to construct the curves in Figures 1 and 2; and the University of London for a grant made to one of us (J. W. F.) to cover the cost of the Unicam spectrophotometer used mainly in this work. Some of this work has been carried out by one of us (G. E. D. H. M.) as part of the requirements for the Ph.D. degree of the University of London.

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## DISCUSSION

The papers on cascara were taken together. The first was presented by MR. T. D. WHITTET and the second by MR. G. E. D. H. MAHRAN.

MR. T. C. DENSTON (London) referred to the variation in activity of different batches of liquid extract of cascara and asked the authors of

the first paper whether they had investigated the effects of time and temperature of autoclaving on their preparations. He wondered whether the method was applicable on a commercial scale.

MR. H. DEANE (Long Melford) said it would be interesting if Mr. Whittet could compare an alcoholic extract with the B.P. extract. Some years ago it was found that treatment of the powdered bark with hydrogen peroxide before extracting with spirit gave an extract which was free from griping properties.

DR. W. MITCHELL (London) said that as both Mr. Deane and himself had pointed out in a paper to the 1948 Conference, the application of a vacuum for drying had little or no effect on the temperature in the final stages of drying, but heating over 100° C. resulted in the extract failing to comply with the B.P. limit test for water-soluble extractive. Did the authors' products comply with that test? The suggestion that concentration took longer under vacuum was surprising. With properly designed equipment the drying process was shortened. That was very desirable in large-scale manufacture.

Turning to the results in Table 1, it was puzzling that the yield of extract by the B.P. 1932 method was lower than that obtained from the same bark by the B.P. 1948 process. It seemed possible that the initial cold water extraction was, for some reason, more efficient in the authors' B.P. 1948 preparation. That might also account for the higher recovery of glycosides as shown by the chemical and biological assays. That seemed more likely than the authors' suggestion that vacuum evaporation had caused greater destruction of glycosides, and in any case vacuum evaporation would not account for the lower yield.

The results did not prove that the Greco and Dumez autoclaving method was more efficient than simple extraction with boiling water. The Bruce method would appear to be quite impracticable for large-scale working, and the fact that it gave a yield similar to the B.P. 1932 method suggested that it was not autoclaving but the subsequent boiling water percolation that made the Greco and Dumez method show better results. He was under the impression that the belief that a griping or emetic principle was present in cascara bark and disappeared on storage was now more or less abandoned. The fact that the ratio between the results of biological and chemical assays was lower in galenicals (presumably made by cold water extraction) than in cascara bark could suggest two things: (*a*) that cold water not only extracted less total anthraquinone glycosides, but also relatively still less of the most biologically active compounds or (*b*) that in the concentration of the original water extracts to give the galenicals, the glycosides were partially inactivated. Had the authors any information on the relative average amounts of free anthraquinones in the bark and in galenicals?

MR. A. W. BULL (Nottingham) asked Mr. Whittet within what order he would expect his results to be reproducible. Did the authors still consider that the variation in the method of concentration would account for the surprising variation in yields shown in Table 1? Many

problems would be involved in operating methods (C) and (D) on a large scale.

MR. BOARDMAN (Manchester) said that apparently the present B.P. method of extraction was not so bad after all. Hot water would improve the extractive yield, but might make it necesssary to reduce the figure for water-soluble extractive to 75 instead of 80 per cent. From experiments which he had carried out he had been unable to convince himself that there was any difference in the extractive from new or old bark.

MR. J. H. OAKLEY (London) pointed out that for the bark, the biological assay result exceeded that of the chemical assay; whereas for the galenical preparation the converse was the case. Both the liquid and the dry extract were prepared by extraction of the bark by cold water as compared with the elixir which was extracted by boiling water in the presence of magnesia. There appeared to be no difference according to the paper, yet he felt convinced that hot extraction was more effective than cold. On a large scale the methods of Whittet and Bruce would be uneconomical. The higher activity of the bark as determined biologically might be due to the digestive system of the animals being better able to extract the active principle. Had Mr. Mahran any information on whether an acid or alkaline extraction medium was more effective than plain distilled water? Was the figure of 3·68 for glycosides present in the bark, obtained by Mr. Whittet, derived by extraction with water and assaying, or by administration of the bark to animals?

MR. R. L. STEPHENS (Brighton) asked Dr. Fairbairn and Mr. Mahran where the loss of activity had occurred in the preparation of the dried extracts. If any biological activity were left in the apparently exhausted bark, that would answer the question, and show that an animal could extract active material which was not extractable by water.

MR. T. D. WHITTET asked whether Dr. Fairbairn and Mr. Mahran had noticed similar anomalous results in B/C ratios with other anthraquinone drugs. The work he had done suggested that high B/C ratios did not depend on efficiency of extraction, since the ratio was inversely proportional to the efficiency of the extraction. In some of Dr. Fairbairn's work with senna he believed that he had found that alcoholic extraction was more efficient than aqueous extraction. He agreed that the difference between the 1932 and 1948 figures was puzzling, and it was being investigated further. One of the difficulties was the criterion for the exhaustion of cascara. Both taste and a test for glycosides had been used but neither was quite satisfactory. He did not think that failure to comply with the B.P. limit test for water-soluble extractive was of great importance; he regarded the test as a temporary expedient for standardising the extract before chemical or biological tests were available. All the extracts were prepared from the same bark, which was assayed by the methods of Fairbairn and Mahran as described in their paper. He agreed that his methods were not applicable on a large scale. Various times and temperatures for autoclaving had been tried, but in his view they were not critical. It might be that the use of boiling water was the critical factor. He considered that the criterion

of a good extract was its activity, and it might be that some preparation of the crude bark would have to be formulated instead of using elaborate extraction methods.

MR. G. E. D. H. MAHRAN, in reply, said there was a difference in the chemical assay of a fresh and an old bark. The fresh bark showed a much larger amount of colouring or interfering substances than the old bark, as confirmed by the final shapes of absorption curves of each. This was being investigated as a method of testing the age of bark, and work was also being carried out to show to what extent the presence of colouring matter may be correlated with the griping effect. The chemical assay could be used to determine the amount of free anthraquinone compounds in bark and galenicals. The amount varied in different samples, but removal by extraction with ether or chloroform did not result in any loss of activity. This supported the theory of Fairbairn that anthracene derivatives are highly active as anthranol glycosides, less active as free anthranols, and much less active as free anthraquinones. The biological activity ran parallel with the content of combined anthraquinone compounds. In senna, biological activity ran parallel with glycosidal content and in rhubarb, with the content of combined rhein-like compounds. It was found that the active principles dissolved without any loss when the infusion was maintained at a temperature approaching 100° C. for 15 minutes followed by the addition of alkali to *p*H 6. He considered that no activity could still be retained in the marc left after preparing a 70 per cent. alcoholic extract of cascara.

With regard to the loss of activity in making galenicals, when carrying out the chemical and biological assays on the bark, the B/C ratio was 1·2; when the extracts were assayed the B/C ratio was found to be only half this value, i.e. 0·6. This was noticed only in cascara. It appeared that there might be a factor lost during the preparation of the galenicals. The presence of an enzyme which might hydrolyse or destroy the glycosides was considered, but it was found that the method of preparation was the cause. The bark was extracted with cold and hot water, and extracts were always found to have a B/C of 0·6. Bark was also extracted with absolute methanol and ethanol but the B/C ratio for the extract remained low. It appeared that there was a factor which was not extracted by water or alcohol alone, and it seemed that the selection of solvents accounted for the phenomenon. By extracting the bark with 70 per cent. ethanol and evaporating the liquid to dryness under vacuum at a temperature not exceeding 40° C., a dry extract was obtained which had a B/C ratio the same as that of the crude drug, i.e. 1·2, and which possessed corresponding activity.