VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART II.—THE EVALUATION OF SENNA POD AND ITS PREPARATIONS

BY J. W. FAIRBAIRN AND I. MICHAELS

From the Pharmacognosy Research Laboratory, School of Pharmacy, University of London, and the Westminster Laboratories, Ltd., London, N.W.1.

Received July 6, 1950

INTRODUCTION

THE quality of senna pod is usually assessed by its appearance, its water-soluble extractive and, to a certain extent, by its geographical source, the Alexandrian pod (*Cassia acutifolia* Delile) being more highly esteemed than the Tinnevelly (*C. angustifolia* Vahl). In a previous paper[1] it was shown that the glycosides (sennosides A and B) rather than the free anthraquinones are the active constituents and hence an estimation of the glycosidal content of a sample of pod should be a reliable indication of its biological activity. Further experiments have confirmed the close relationship between glycosidal content and biological activity and the results of these comparative experiments are recorded in this paper. The analyses of a large number of commercial samples of senna pod have shown that the above mentioned criteria of appearance and water-soluble extractive are no guide to the glycosidal content, though there is justification for considering the Alexandrian pod to be better than the Tinnevelly (see Part III).

The method used for estimating the glycosidal content was based on that of Kussmaul and Becker[2] and a detailed account of the modified chemical assay process which has been developed is given in this paper.

THE CHEMICAL ASSAY OF SENNA POD

Extraction of the glycosides. As the chief object has been the assay of the crude drug and its preparations, it has been essential to devise a reliable means of extracting the glycosides completely for assay purposes; the rest of the process is similar to that of Kussmaul and Becker except for alteration in details. For the sake of completeness a detailed account of the whole assay process is included. As a preliminary, the crude drug in fine powder was used to determine the true glycosidal content by hydrolysing a suspension directly; this was obviously necessary as a basis for comparative work. The use of a suspension of the powdered drug, however, is inconvenient for regular assay purposes and is inapplicable when whole or coarsely powdered pod is being dealt with; a suitable extraction process, therefore, is an essential stage.

Though the glycosides are sparingly soluble in water they dissolve readily in the presence of alkali, due to the formation of soluble salts;

it was therefore decided to use water as a solvent and to adjust the *p*H suitably. Hot water will penetrate the tissues of the pod more rapidly than cold water and thereby facilitate solution, but because of the somewhat thermolabile nature of the glycosides prolonged exposure to high temperature must be avoided.

Table I summarises the results obtained by assaying 2 per cent. infusions prepared by infusing 10 g. of pod in coarse powder under various conditions. It is seen that the active principles are incompletely soluble in water alone, but dissolve entirely and without loss when the infusion is maintained at a temperature approaching 100°C. for 10 to 15 minutes followed by the addition of alkali to *p*H 6 to 7. Immersion in a boiling water bath for less than 10 minutes may be sufficient for complete extraction, whereas immersion for longer than 20 minutes results in gradual decomposition of the active principles.

TABLE I

EFFICIENCY OF VARIOUS INFUSION TECHNIQUES FOR THE EXTRACTION OF GLYCOSIDES FOR THE ASSAY OF SENNA POD

Method of preparation of infusion			Proportion of active principles present
Vehicle	Procedure	Length of time of infusion	
			Per cent.
Cold water ...	... Shaken occasionally ...	24 hours	84
Boiling water	... Immersed in boiling water bath; cooled and adjusted to volume.	15 minutes	75·0
		20	75·0
Boiling water	... Immersed in boiling water bath; adjusted to *p*H 6 to 7; cooled and adjusted to volume.	5 minutes	83·4
		10	100
		15	100
		20	100
		30	85·6

Preliminary experiments showed that, in order to reduce sampling errors to a minimum, it is necessary to use a larger amount of whole pod and pod in coarse powder than when pod in fine powder is to be assayed.

The standard procedures for preparing infusions of senna pod which have been developed for chemical assay are as follows.

(a) *Whole pod.* 10 g. is cut into strips 2 to 3 mm. wide and transferred to a 500-ml. graduated flask. About 450 ml. of boiling water is added, the flask is immersed in a boiling water-bath for 10 minutes and frequently agitated. After removal from the bath, sufficient N sodium hydroxide is added to adjust to *p*H 6 to 7. The infusion is immediately cooled and made up to 500 ml.

(b) *Pod in coarse powder.* 10 g. is infused in about 450 ml. of boiling water for 10 minutes, the *p*H is adjusted to 6 to 7 as in (a), and the volume made up to 500 ml.

(c) *Pod in fine powder.* 1 g. is infused in about 90 ml. of boiling water for 10 minutes, the *p*H is adjusted to 6 to 7 as in (a) and the volume made up to 100 ml.

In every instance 10 ml. of the filtered infusion is taken for assay.

The Assay Process

(i) *Removal of the free anthraquinones.* N hydrochloric acid is added to adjust the 10 ml. of infusion to about *p*H 3, which is then shaken with 60 ml. and then 40 ml. quantities of ether until the ether extract is colourless. The combined ether fractions are washed with small quantities of acidified water and the washings added to the original aqueous solution. This solution contains only glycosides and no free anthraquinones.

(ii) *Hydrolysis of the glycosides.* The aqueous solution is heated with half its volume of 10N sulphuric acid in a boiling water bath for 15 minutes. The aglycone separates as a brown flocculent precipitate on cooling.

(iii) *Extraction and purification of the aglycones.* The liquid is shaken with 80 ml. of ether and allowed to settle; the aqueous portion is separated and the yellow ether solution 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 30 per cent. sodium hydroxide solution and to this solution is added the separated aqueous portion (which contains excess of acid). The extraction is continued with 40 ml. quantities of ether in a similar manner, until the ether fraction is colourless, indicating complete extraction of the aglycone.

In order to separate those aglycones derived from the sennosides A and B, i.e., those containing carboxyl-groupings on the anthracene nucleus, the combined ether portions are extracted repeatedly with small volumes of N sodium bicarbonate until an extract is colourless. To the combined bicarbonate solutions ether is added, the solution is acidified with 50 per cent. sulphuric acid and, when effervescence has ceased, the mixture is shaken to transfer the aglycones to the ethereal layer. The aqueous layer is run off and the yellow ethereal solution filtered into a graduated flask. Any brown residue is dissolved in sodium hydroxide solution as before, re-acidified and the extraction repeated with 20 ml. quantities of ether. The solution is finally adjusted to volume with ether.

(iv) *Colorimetric estimation.* A suitable volume of the aglycone solution in ether is extracted by shaking with small portions of N caustic soda solution. To the combined alkali solutions 0·2 ml. of 3 per cent. hydrogen peroxide solution per 10 ml. of alkaline liquid is added and the mixture heated in a boiling water-bath for 4 to 5 minutes cooled, made up to a suitable volume with N caustic soda, and the intensity of the purple-red colour determined colorimetrically. For this purpose, three types of instruments have been used, viz., the EEL colorimeter (a simple instrument with a single photocell connected to a sensitive galvanometer); the *Spekker Absorptiometer* (with two balanced photocells and a null-point reading on the galvanometer) and the *Unicam Diffraction Grating Spectrophotometer* (with a single photocell connected to a sensitive galvanometer). In every instance calibration curves were made using pure sennosides A and B, and from these a final curve to represent a

mixture of equal parts of the sennosides was constructed; the peak of the wavelength of the incident light used was 520 mμ.

Limits of Accuracy of the Assay Process

The limits of accuracy of the assay process were estimated by carrying out a number of replicate assays on infusions prepared by the standard procedures from the drug (a) in fine powder (1 g. of drug in 100 ml.), and (b) in coarse powder (10 g. of drug in 500 ml.), and (c) on infusions prepared from the whole pod (10 g. in 500 ml.). The results are recorded in Table II and indicate that with (a) fine powder, (b) coarse powder and (c) whole pod, the coefficients of variation are 1·3 per cent., 5·71 per cent. and 6·14 per cent. respectively, which mean that for a probability of P = 0·99, the results of assays for the fine powder would be within 4 per cent. of the true value, whereas those for the coarse powder and whole pod would be within 17 to 18 per cent. of the true value. The results from duplicate assays of an infusion carried out on the same day, were frequently identical and never deviated from each other by more than 5 per cent. It is likely that the limits of error in the assay of whole pod and coarse powder could be decreased by using a sample larger than 10 g.

These figures show that, apart from sampling errors, the chemical assay process described is accurate to within ± 4 per cent.

TABLE II

Limits of accuracy of the chemical assay process when applied to (a) pod in fine powder, (b) pod in coarse powder, and (c) whole pod

	Fine powder	Coarse powder	Whole pod
	Glycosides/g of drug	Glycosides/g. of drug	Glycosides/g. of drug
	32·5 mg.	30·5 mg.	30·8 mg.
	32·5	33·5	33·8
	31·8	29·5	31·2
	32·2	31·8	28·8
	31·2	31·1	31·1
	31·5	28·5	28·4
Mean	32·0 mg./g.	30·8 mg./g.	30·7 mg./g.
Coefficient of variation	1·30 per cent.	5·71 per cent.	6·14 per cent.

Note.—The coarse powder and whole pod represented the same sample, whereas the fine powder was made from a different sample.

Correlation Between Biological Activity and Glycosidal Content

In Table III are set out the results of comparative assays of biological activity and glycosidal content of various samples of pod and simple dilutions. The glycosidal content was determined by the chemical assay process already described and the biological activity by Lou's[3] process. These results reveal a remarkably high degree of correlation and indicate that the chemical assay is a reliable method for the evaluation of the pod, but do not necessarily prove that the activity of the crude drug is entirely

due to the sennosides. However, for different samples of pod, the relative activities can be reliably assessed by means of the chemical assay.

TABLE III

BIOLOGICAL ACTIVITY AND GLYCOSIDAL CONTENT OF SENNA POD

Sample	Biological activity		Glycosidal content as sennosides A+B determined chemically	Ratio of Biological activity to Glycosidal content
	P_S =100	As sensosides A+B		
		Per cent.	Per cent.	
Standard Pod, P_S ...	100	3·200	3·20	1·00
Powdered pod	(i) 148 (ii) 122 } Mean=135	4·320	4·60	0·94
Powdered pod, mixed with inert material.	(i) 15·1 (ii) 14·6 } Mean=15·0	0·480	0·475	1·01
Powdered pod, mixed with inert material.	(i) 16·6 (ii) 20·3 (iii) 13·2 } Mean=16·7	0·534	0·523	1·02
Powdered pod	(i) 86 (ii) 95 } Mean=91	2·910	2·830	1·03

Note.—The biological assay results have been expressed in relation to the Standard Pod, P_S=100 ; as this sample contains 3·2 per cent. of sennosides A and B, the biological assay results have been expressed in terms of sennosides A+B in order to compare with the chemical assay results.

THE EFFECT OF INTERFERING SUBSTANCES ON THE CHEMICAL ASSAY PROCESS

It was noticed that when certain non-official preparations of senna pod were assayed biologically and chemically, the ratios between biological activity and glycosidal content, as shown in Table IV, were much higher than those given from the comparative assays of pod described above. It was inferred that ingredients were present which interfered with the extraction of the anthracene derivatives in the colorimetric assay process. That liquorice interferes with the Bornträger reaction has already been noted[4], and until this point is clarified it is recommended that the chemical assay process be used only to evaluate samples of the crude drug and its simple preparations, and the bio-assay for those preparations of senna pod, the exact composition of which is not known.

TABLE IV

BIOLOGICAL ACTIVITY AND GLYCOSIDAL CONTENT OF NON-OFFICIAL PREPARATIONS OF SENNA POD

Description	Biological activity		Glycosidal content as sennosides A+B determined chemically	Ratio of Biological activity to Glycosidal content
	P_S=100	As sennosides A+B		
		Per cent.	Per cent.	
A liquid preparation...	(i) 9·9 (ii) 14·3 } Mean=12·1/ml.	0·387 $^{W}/_{v}$	0·300 $^{W}/_{v}$	1·29
A liquid preparation...	(i) 17·4 (ii) 13·6 } Mean=15·5/ml.	0·500 $^{W}/_{v}$	0·340 $^{W}/_{v}$	1·48
A pastille	(i) 10·5 (ii) 11·1 } Mean=10·8/g.	0·346 $^{W}/_{w}$	0·248 $^{W}/_{w}$	1·40

Note.—See Table III.

SUMMARY

1. A technique is described for preparing an infusion of senna pod (whole and in coarse and fine powder) containing all the active glycosides.

2. Details are given of a method, based on that of Kussmaul and Becker[2], for estimating the glycosides colorimetrically; the method is accurate to within ± 4 per cent.

3. A high degree of correlation has been shown to exist between the biological activity and glycosidal content, indicating that the chemical assay is a reliable method for evaluation of senna pod.

4. It has been recommended that since other ingredients, such as liquorice, may interfere with the chemical assay process, the bio-assay be used for estimating the activity of senna pod preparations. the exact composition of which is not known.

ACKNOWLEDGMENTS

We would like to thank the Directors of Westminster Laboratories, Ltd., for certain facilities generously provided during the course of this investigation, and for permission for one of us (I.M.) to publish the results. We would also like to thank Mr. T. C. Lou and Mr. M. R. I. Saleh for carrying out the bio-assays.

REFERENCES

1. Fairbairn, *J. Pharm. Pharmacol*, 1949, **1**, 683.
2. Kussmaul and Becker, *Helv. chim. Acta*, 1947, **30**, 59.
3. Lou, *J. Pharm. Pharmacol.*, 1949, **1**, 673.
4. Fairbairn, *Pharm. J.*, 1942, **156**, 381.