VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART III. GALENICAL PREPARATIONS OF SENNA POD

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In Part I of this series of papers[1] reference was made to a preliminary test on an extract of senna pod which was found to contain only about 5 per cent. of its theoretical activity. Biological and chemical tests have since been carried out on a number of galenical preparations of senna pod and the results have confirmed that only a small proportion of the activity of the pod is present in these concentrated preparations. The ultimate aim of the work described in this paper has been to devise a method of preparing an extract representing the full activity of the pod from which it was made, and as a result, it has been possible to set out the principles underlying efficient extraction and concentration processes.

EVALUATION OF SAMPLES OF SENNA POD

To assess the efficiency of an extraction and concentration process it is necessary to know the glycosidal content and water-soluble extractive of the pod. Table I summarises the results obtained from the examination of a number of samples of Alexandrian (*Cassia acutifolia*, Delile) and Tinnevelly (*C. angustifolia*, Vahl) pod of manufacturing and hand-picked grades. The glycosidal content was determined by the assay process described in Part II[2] and the water-soluble extractive by the B.P. method. Moisture content determination was carried out by drying at 100°C. in an oven. These figures show that (a) appearance is not a reliable method of evaluating the pod, as the best quality hand-picked Alexandrian pod sometimes has a lower glycosidal content than the dark and much broken manufacturing grade; (b) the average glycosidal content and range for Alexandrian pod was 2·37 to **3·22** to 4·34 per cent., and for Tinnevelly 1·22 to **1·96** to 2·78 per cent.; (c) the average water-soluble extractive and range for Alexandrian pod was 25·8 to **28·3** to 31·2 per cent. and for Tinnevelly 20·7 to **23·0** to 25·5 per cent.; the Alexandrian pod contains more activity and water-soluble extractive than the Tinnevelly, but within each species no correlation was apparent between glycosidal content and water-soluble extractive. It is interesting to note that not all samples of Alexandrian pod and none of the samples of Tinnevelly pod examined complied with the B.P. requirement of containing not less than 28 per cent. of water-soluble extractive. The B.P.C., on the other hand, states that senna fruit has a water-soluble extractive of 18 to 30 per cent.; the extractives of all samples exceeded the lower

limit, and in three samples of Alexandrian pod the upper limit was exceeded. Since no indication is available for ascertaining the source of pod used in commercial preparations, 2·59 per cent. (average of 3·22 and 1·96 per cent.) has been taken as the "average glycosidal content of senna pod," and 25·65 per cent. (average of 28·3 and 23·0 per cent.) as the "average water-soluble extractive."

TABLE I

THE POTENCY OF COMMERCIAL SAMPLES OF SENNA POD

Sample	Source	Grade	Year purchased	Glycosidal content as sennosides A and B per cent.	Water-soluble extractive, per cent.	Moisture content, per cent.
1	Alexandrian	Hand-picked	1946	3·20	—	—
2	,,	,,	1949	2·37	27·1	10·0
3	,,	,,	1950	3·05	26·7	10·5
4	,,	Manufacturing	1949	4·34	30·1	8·5
5	,,	,,	1949	3·32	31·2	8·3
6	,,	,,	1949	2·83	26·8	10·2
7	,,	,,	1950	3·07	28·9	8·4
8	,,	,,	1948	2·78	30·2	10·4
9	,,	,,	1950	2·91	27·2	9·9
10	,,	,,	1950	3·78	28·6	10·2
11	,,	,,	1949	3·28	26·9	8·7
12	,,	,,	1946	2·98	29·6	10·0
13	,,	,,	1949	3·21	28·5	9·0
14	,,	,,	1949	3·89	25·8	—
15	,,	,,	1950	3·30	28·9	—
16	Tinnevelly	Hand-picked	1950	2·78	23·3	9·8
17	,,	,,	1950	2·64	23·7	9·8
18	,,	Manufacturing	1950	1·63	20·7	8·8
19	,,	,,	1949	1·29	21·3	11·5
20	,,	,,	1949	2·23	25·4	9·6
21	,,	,,	1950	1·35	23·3	10·3
22	,,	,,	1949	2·55	25·5	11·0
23	,,	,,	1950	1·22	20·8	10·5

THE POTENCY OF COMMERCIAL SAMPLES OF LIQUID EXTRACT OF SENNA B.P.

Liquid extract of senna B.P. (which is prepared by a triple maceration process with cold water) is a 1:1 extract, *i.e.*, 1 ml. should represent the activity of 1 g. of pods. Samples of this galenical prepared by a number of manufacturers were purchased on the open market and were assayed for glycosidal content and for total solids. The values are recorded in Table II, as the potencies found experimentally and as a proportion of the glycosidal content of the "average" pod in order to indicate the efficiency of the complete process of manufacture and storage. Samples A to F were assayed within a month of purchase, G and H six months after purchase. It is seen that less than 5 per cent. of the original activity exists in the majority of these extracts; even if one of the poorest grades of Tinnevelly pod (possessing, *e.g.*, 1·22 per cent. glycosides) had been used, the proportion of glycosides in the best sample assayed would be only 17 per cent. of that in the pod. The total solids content of the extracts is very low when compared with the average water-soluble extractives of the pod, even taking into account that proteinous matter is precipitated and removed during preparation. These figures suggest that the pharmacopœial process of triple maceration does not allow complete

extraction; this fact could be responsible, partly or completely, for the remarkably low glycosidal contents of the commercial extracts.

TABLE II

POTENCY OF COMMERCIAL SAMPLES OF LIQUID EXTRACT OF SENNA, B.P.

Manufacturer	Glycosidal content	Proportion of theoretical glycosidal content	Total solids
	per cent. w/v	per cent.	per cent. w/v
A	0·210	8·1	14·3
B	0·170	6·6	14·6
C	0·087	3·3	11·1
D	0·080	3·1	15·4
E	0·068	2·6	11·4
F	0·057	2·2	15·8
G	0·030	1·2	10·7
H	0·027	1·0	24·0

Note—Liquid extract of senna, B.P. should contain in 1 ml. the activity of 1 g. of pod. If the average glycosidal content of the pod is assumed to be 2·59 per cent., liquid extract of senna should contain 2·59 per cent. w/v of glycosides. This figure has been used in calculating the values in column 3.

THE POTENCY OF NON-OFFICIAL PREPARATIONS OF SENNA POD

For reasons already stated (see Part II[2]), non-official preparations of senna pod were assayed biologically. Table III records the results of the bio-assays of several preparations. The reputed activity has been calculated on the assumption that a pod of average potency had been used in the preparation; by comparing the theoretical values with those found experimentally, the proportion of glycosides surviving the manufacturing process has been computed. The results indicate that the proportion of the theoretical glycosidal content is higher than that in pharmacopœial preparations; this may be due to (a) the use of a better quality pod than the "average", (b) more care being taken to standardise and stabilise

TABLE III

BIOLOGICAL ASSAYS OF NON-OFFICIAL PREPARATIONS OF SENNA POD

	Description	Reputed activity	Biological activity	Proportion of theoretical glycosidal content
		Per cent. glycosides	Per cent. glycosides	Per cent.
1	A liquid	1·295 w/v	0·387 w/v	30·7
2	A liquid	1·295 w/v	0·500 w/v	38·6
3	A pastille	0·856 w/w	0·346 w/w	40·5
4	Granules	0·500 w/w	0·534 w/w	107·0

Note—The biological activity has been expressed in relation to the Standard Pod, Ps, which contains 3·20 per cent. of sennosides A + B.

the product, and (c) the presence of other ingredients which may have a synergistic effect on the purgative activity of the glycosides.

The Potency of Commercial Samples of Concentrated Infusion of Senna B.P.

Concentrated infusion of senna, B.P. is prepared by a reserved percolation process using alcohol (20 per cent.) and should be eight times as active as fresh infusion of senna, B.P.C. Samples of this galenical prepared by the same manufacturers as those listed in Table II, were analysed. The results, recorded in Table IV, show once more that there was little activity present. It was therefore necessary to establish the glycosidal content of the fresh infusion in order to be able to compute the theoretical potency of the concentrated infusion. Results of experiments recorded later show that the B.P.C. fresh infusion contains approximately 1·75 mg. of glycosides per ml.; the concentrated infusion should therefore contain 14 mg. per ml.

TABLE IV

Potency of commercial samples of concentrated infusion of senna B.P.

Manufacturer	Glycosidal content	Proportion of theoretical glycosidal content	Total solids
	Per cent. w/v	Per cent.	Per cent. w/v
A	0·076	5·58	7·1
B	0·026	1·85	9·4
C	0·060	4·29	11·1
D	0·073	5·21	15·9
E	0·012	0·86	11·9
F	0·054	3·86	14·5
G	0·035	2·50	9·3
H	0·210	15·00	16·4

Note—Concentrated infusion of senna, B.P. should have eight times the activity of fresh infusion of senna, B.P.C. The latter preparation has been found to contain approximately 1·75 mg. of glycosides per ml.; concentrated infusion of senna should therefore contain approximately 1·4 per cent. w/v of glycosides. This figure has been used in calculating the values in column 3.

Infusions of Senna Pod

(a) *Simple Infusions.* The B.P.C. 1949, p. 797, states that a simple infusion may be made by soaking 4 to 12 pods in about 5 fl. oz. of warm water for about 12 hours. As the mean weight of a pod is given as 0·16 g. the maximum recommended concentration is approximately 12 in 1,000. On packets of pods bought from pharmacies, in some instances the directions recommend that pods be soaked in cold water and in others in hot water, for periods varying from 3 to 4 hours to overnight. Infusions were prepared by adding 1,000 g. of (i) boiling water, (ii) water at 50°C. and (iii) cold water, to 12 g. samples of best quality hand-picked Alexandrian pod (containing 3·05 per cent. of glycosides) and assayed after varying periods of time. The results are presented in Table V. It

has previously been shown (Part II)[2] that not more than approximately 4/5 of the active glycosides in the pod can be dissolved by water alone. It is seen, therefore, that a 1·2 per cent. infusion made with boiling water extracts in 15 minutes the maximum possible activity; water at 50°C., and cold water, even after 24 hours, extract sub-maximal amounts. If it is assumed that a simple infusion extracts approximately 3/4 of the activity of the pod, then 5 fl. oz. of an infusion made from 4 to 12 pods would therefore contain about 15 to 45 mg. of glycosides.

(b) *Fresh Infusion of Senna B.P.C.* The fresh infusion was prepared using 50 g. of the sample of pod used in (a) and 500 g. of boiling water. The preparation was assayed after the prescribed infusion time and after further periods. A similar infusion made with 25 g. of pod in 500 g. of water was also prepared and assayed. The results (Table V) show that in the B.P.C. infusion, 53 per cent. of the activity of the pod has been extracted after 15 minutes and that no more active principles are extracted even after 1 hour's contact. With the 5 per cent. infusion, 65 per cent. of the pod's activity has been extracted. The construction of the B.P.C. fresh infusion was approximately 1·75 mg. of glycosides per ml. The official dose range of 15 to 60 ml. would therefore offer 21·5 to 105 mg. of glycosides; this is considerably in excess of that administered in a simple infusion of from 4 to 12 pods and also of the B.P. dosage of senna pod (*viz.* 0·6 to 2 g., representing approximately 18 to 60 mg. of glycosides).

TABLE V

POTENCY OF INFUSIONS OF SENNA POD AND EFFICIENCY OF EXTRACTION PROCESS

Method of preparation			Concentration of infusion	Proportion of active principles extracted
Vehicle	Proportion of pod Per cent.	Length of time of infusion Hours	Glycosides mg./ml.	Per cent.
Boiling water ...	1·2	¼	0·298	81·5
		24	0·307	84·0
Water at 50°C. ...	1·2	4	0·258	71·0
		24	0·258	71·0
Cold water ...	1·2	24	0·263	72·0
Boiling water ...	5·0	¼	0·990	65·0
Boiling water ...	10·0	¼	1·69	53·0
		½	1·69	53·0
		1	1·69	53·0

The results, so far, indicate that, whereas fresh infusions of senna contain approximately 1/2 to 3/4 of the activity of the pod, commercial samples of concentrated preparations such as liquid extract of senna and concentrated infusion of senna contain, at the most, about 1/10 of the activity. The fresh infusion therefore represents the most active of the official galenicals. The low glycosidal content of the concentrated preparations suggests that in addition to incomplete extraction, losses may occur during evaporation and possibly during subsequent storage. Thus, in order to locate the stages at which losses in activity take place, it is

necessary to investigate the efficiency of the extraction processes of the B.P. (triple maceration, reserved, and also general percolation), followed by studies of the effect of heat on the glycosidal content of aqueous macerates and percolates of senna pod.

The Efficiency of the Pharmacopœial Processes for the Extraction of Senna Pod

(a) *Triple Maceration.* The B.P. triple maceration process for liquid extract of senna was carried out using pod containing 3·07 per cent. of glycosides and 28·9 per cent. of water-soluble extractive. Each macerate was assayed for glycosides and total solids. The results are submitted in Table VI and show that only about 2/3 of the active principles and 3/4 of the water-soluble extractive have been extracted by the process. These figures help to explain, in some measure the low glycosidal content of commercial preparations of liquid extract of senna; nevertheless the discrepancy is still great and suggests that activity is also lost during the subsequent concentration stage and possibly during storage.

TABLE VI

Efficiency of triple maceration extraction process for preparation of liquid extract of senna, B.P.

Macerate	Volume	Proportion of glycosides extracted	Proportion of total solids extracted
	ml.	per cent.	per cent.
1st	400	34	37
2nd	390	17	22
3rd	430	13	18
Pressings ...	130		
Totals ...	1350 ml.	64	77

Weight of pod used = 200 g.

(b) *Reserved Percolation.* Concentrated infusion of senna, B.P. was prepared, using a sample of pod in coarse powder containing 3·08 per cent. of glycosides and 35·3 per cent. of water-soluble extractive. Each of the percolates and the finished preparation was assayed for glycosides and total solids. (The second percolate was concentrated by heating under reduced pressure at a temperature which never exceeded 40°C.) The results are submitted in Table VII. Since approximately half of the activity of the concentrated infusion is derived from the reserved percolate (which in the above preparation had a potency of 0·76 per cent. w/v of glycosides), even if all the activity in the second percolate were destroyed by overheating during concentration, the finished product should not contain less than about 3/4 of this value. The freshly prepared concentrated infusion contained 1·02 per cent. w/v of glycosides, indicating that there had been no loss during concentration of the second percolate. The proportion of the theoretical glycosidal content (see note

to Table IV) was therefore 73 per cent. However, the concentrated infusion contained only 6 instead of 8 times the activity of the fresh infusion. This discrepancy exists because the pharmacopœial directions for the preparation of the concentrated infusion permit the extraction of only about 43 per cent. of glycosides, whereas in the preparation of the fresh infusion approximately 53 per cent. is extracted (Table V). The remarkably low glycosidal content of commercial samples suggests that deterioration takes place subsequent to manufacture during storage.

TABLE VII

EFFICIENCY OF RESERVED PERCOLATION PROCESS FOR PREPARATION OF CONCENTRATED INFUSION OF SENNA B.P.

Percolate fraction	Proportion of glycosides extracted	Proportion of total solids extracted
	per cent.	per cent.
0 to 140 ml.	21·7	35
140 to 340 ml.	21·0	31
Totals	42·7	66

Weight of pod used = 160 g.

(c) *General Percolation Process.* Senna pod in coarse powder containing 3·08 per cent. of glycosides and 35·3 per cent. of water-soluble extractive was percolated with chloroform water as in the B.P. general process. The percolate was collected in fractions which were assayed for glycosides and total solids. The results, recorded in Table VIII, show that with a percolate : drug ratio of 15, all the soluble extractive but only 3/4 of the active principles are extracted, and that practically the whole of this quantity is contained in the first six volumes of percolate.

PROPORTION OF GLYCOSIDES TO WATER-SOLUBLE EXTRACTIVE (OR TOTAL SOLIDS)

The proportion of glycosides to water-soluble extractive or total solids is useful and important, as it offers a ready means of ascertaining the efficiency of the extraction and concentration processes. For the sample of pod used above the value is 87 mg./g., which means that if the active principles were extracted completely with water and the solution evaporated to dryness without loss, the glycosidal content of the product would be 87 mg./g.

EFFECT OF HEAT ON THE GLYCOSIDAL CONTENT OF AQUEOUS PERCOLATES OF SENNA POD

The usual method of concentration is evaporation by heat, and accordingly time-temperature effects on aqueous percolates of senna pod were studied. Portions of a percolate (the reaction of which is usually *p*H 5·1 to 5·2) were heated at different temperatures for various periods of time and the loss in glycosidal content was determined. The results.

recorded in Table IX, show that the deterioration is less at lower temperatures and for shorter periods of time as might be expected. By using reduced pressure, evaporation at temperatures between 50° and 60°C.

TABLE VIII

POTENCY OF SUCCESSIVE FRACTIONS OBTAINED BY PERCOLATION OF SENNA POD IN COARSE POWDER WITH WATER

Percolate fraction in ml.	Proportion of glycosides extracted	Proportion of total solids extracted
	per cent.	per cent.
0 to 300	63	85
300 to 600	10	12
600 to 900	1	2
900 to 1500	1	1
Totals	75	100

Weight of pod used = 100 g.

is fairly convenient on a manufacturing scale, but even at this temperature there was a loss of ½ to 1/5 of the initial activity during an 8-hour period of heating. The effect of increasing alkalinity was studied by adjusting portions of a percolate to *p*H values between 5·1 and 8·4, heating at 60°C. for various periods and determining the glycosidal content.

TABLE IX

EFFECT OF HEAT ON GLYCOSIDAL CONTENT OF SENNA PERCOLATE (*p*H 5.1)

Temperature	Period of heating	Proportion of initial glycosidal concentration, per cent.
100°C.	0 minutes	100·0
	5	91·0
	10	84·5
	15	70·4
	30	56·3
	60	53·4
	120	28·2
80°C.	0 hours	100·0
	1	86·0
	2	76·6
	3½	42·9
	6½	23·0
70°C.	0 hours	100·0
	1	92·4
	4	81·0
	6	57·6
	8	51·5
60°C.	0 hours	100·0
	4	82·6
	7½	64·0
	16	60·5
	24	44·2
	48	18·1
50°C.	0 hours	100·0
	2	88·8
	8	81·0
	15	76·0
	24	70·4
	48	67·5
	96	35·2

As the results submitted in Tables IX and X and shown graphically in Figures 1 and 2 indicate, at values more alkaline than *p*H 7·1, there was a striking and rapid loss of activity in comparatively short periods of time.

TABLE X

EFFECT OF HEAT AT 60°C. ON THE GLYCOSIDAL CONTENT OF SENNA PERCOLATE AT DIFFERENT *p*H VALUES

*p*H	Period of heating	Proportion of initial glycosidal concentration, per cent.
6·00	0 hours	100·0
	2	75·0
	4	63·0
	7½	49·0
	16	29·7
	24	23·0
7·10	0 hours	100·0
	2	76·0
	6	30·2
	7½	20·0
8·38	0 hours	100·0
	1	47·0
	2	26·5
	4	12·5

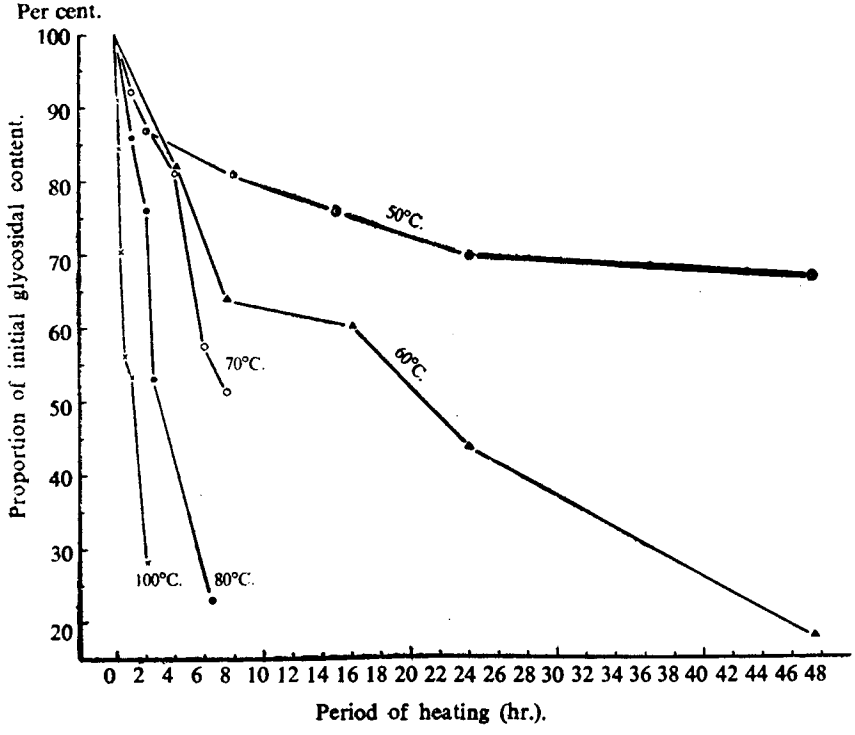

FIG. 1. Effect of heat on glycosidal content of senna pod percolate (*p*H 5·1) (Table 9).

Preparation of Potent Extracts by Concentration of Aqueous Percolates

(a) *A Soft Extract.* A senna pod percolate containing 95·5 mg. of glycosides per g. of total solids was evaporated to a soft extract under reduced pressure at a temperature not exceeding 40°C. The evaporation took 10 hours. The product was assayed and found to contain 56 mg. of glycosides per g. of extract and 93·3 mg. of glycosides per g. of total solids, indicating that 98 per cent. of the activity of the percolate had survived the concentration process.

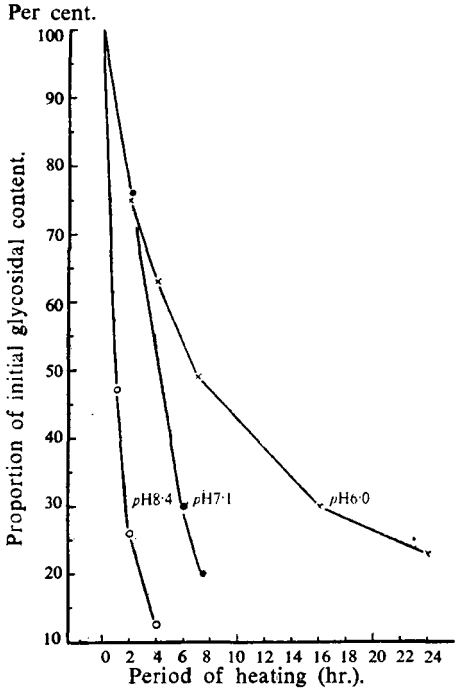

FIG. 2. Effect of heat at 60°C. on senna pod percolate at different *p*H values (Table 10).

(b) *A Liquid Extract.* A percolate containing 83 mg. of glycosides per g. of total solids was heated at 80°C. for 3 minutes, cooled rapidly, set aside for 48 hours to allow proteinous matter to settle, and the liquid decanted. The percolate, when reassayed, showed no loss in activity. It was then concentrated by heating under reduced pressure at a temperature not exceeding 45°C. to a thick liquid (the specific gravity of which was 1·21), which contained 33·4 mg. of glycosides per g. of extract, and 74 mg. of glycosides per g. of total solids, indicating that 89 per cent. of the activity of the percolate had survived the concentration process.

To this extract 25 per cent. of alcohol (90 per cent.) was added (as in the preparation of liquid extract of senna B.P.). The finished preparation was assayed and found to contain 34·8 per cent. of total solids and 2·5 per cent. w/v of glycosides.

Extraction of Senna Pod with Organic Solvents and their Aqueous Dilutions

According to Stoll *et al.*[3] the active principles, sennosides A and B, exist in the crude drug as water-soluble salts and as free glycosides which are almost insoluble in cold water. This explains why it is practically impossible to extract the glycosides completely with water, unless hot water with traces of alkali are used (as in the standard assay process, Part II[2]). Stoll also states that sennoside A is only sparingly soluble in ethyl alcohol, and slightly soluble in methyl alcohol and acetone; sennoside B is more readily soluble in these solvents. However, if the solvents

are diluted to contain about 30 per cent. of water, they then readily dissolve both of the sennosides and their salts. These facts have been confirmed by shaking known amounts of pod in coarse powder with the above-mentioned pure solvents and their aqueous dilutions and assaying the filtered solutions; isopropyl alcohol and its dilutions were also used. The results clearly demonstrated that the solvent action for the glycosides is at a peak when 30 to 40 per cent. of water is present.

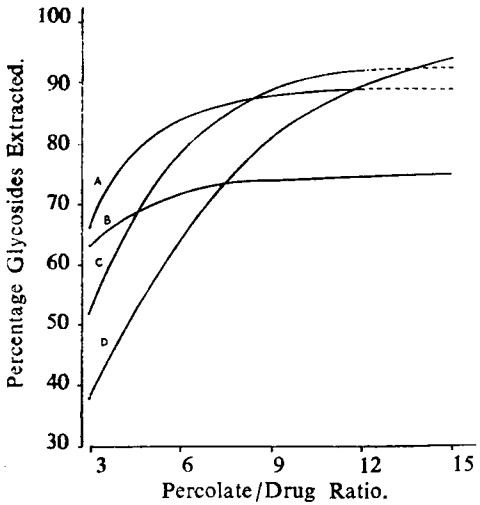

FIG. 3. Extraction of glycosides of senna pod, in coarse powder, by percolation with aqueous dilutions of organic solvents. A, 70 per cent. methyl alcohol; B, 70 per cent. acetone; C, water; D, 70 per cent. ethyl alcohol.

In order to investigate the efficiency of these solvents for the extraction of senna pod, the drug in coarse powder was percolated with 70 per cent. dilutions with water of acetone and ethyl, methyl, and isopropyl alcohols. The proportions of glycosides and total solids extracted in successive fractions of percolates were determined and the results submitted in Table II and expressed graphically in Figures 3 and 4. The results for percolation with water (see Table VIII) are also included for comparison. Percolation with 20 per cent. ethyl alcohol gave results

TABLE XI

POTENCY OF SUCCESSIVE FRACTIONS OBTAINED BY PERCOLATION OF SENNA POD IN COARSE POWDER WITH AQUEOUS DILUTIONS OF CERTAIN ORGANIC SOLVENTS

Weight of pod used = 100 g.

Percolate fraction	70 per cent. v/v acetone		70 per cent. v/v ethyl alcohol		70 per cent. v/v methyl alcohol		20 per cent. v/v ethyl alcohol	
	Proportion of glycosides extracted	Amount of total solids extracted	Proportion of glycosides extracted	Amount of total solids extracted	Proportion of glycosides extracted	Amount of total solids extracted	Proportion of glycosides extracted	Amount of total solids extracted
ml.	per cent.	g.	per cent.	g.	per cent.	g.	per cent.	g.
0 — 300	52·0	16·5	38·7	16·2	61·3	20·8	59·4	27·1
300 — 600	27·1	5·6	27·6	5·1	23·8	4·6	9·3	3·8
600 — 900	10·0	2·0	16·4	2·0	4·0	1·3	1·6	0·9
900 —1200	3·3	1·0	7·9	1·1	1·0	0·6	0·2	0·5
1200 —1500	—	—	4·8	0·7	—	—	—	—
1500 —1800	—	—	2·7	0·5	—	—	—	—
Totals ...	92·4	25·1	98·1	25·6	90·1	27·3	70·5	32·3

almost identical with those for water. (Percolation with 70 per cent. *iso*propyl alcohol failed to extract the active principles to the same extent as the other solvents and the results have therefore not been included.)

The diagrams clearly show that the drug can be exhausted almost completely by 70 per cent. dilutions of acetone, ethyl alcohol and methyl alcohol at a drug: percolate ratio of 1:15. Even at a ratio of 1:9, 80 to 90 per cent. of the glycosides have been extracted; moreover these solvents extract less solid matter than does water. These combined effects increase the proportion of glycosides to total solids in the percolate to values considerably in excess of that given by aqueous percolates and yield solutions from which extracts of much higher potency can be made. The proportion of glycosides to total solids in percolates using 70 per cent. dilutions of acetone, ethyl alcohol and methyl alcohol, ethyl alcohol (20 per cent.), and water, were as follows: 113·5, 117, 101, 67 and 68 mg./g. respectively.

Preparation of Potent Extracts by Concentration of Percolates Made with 70 per cent. Dilutions of Certain Organic Solvents

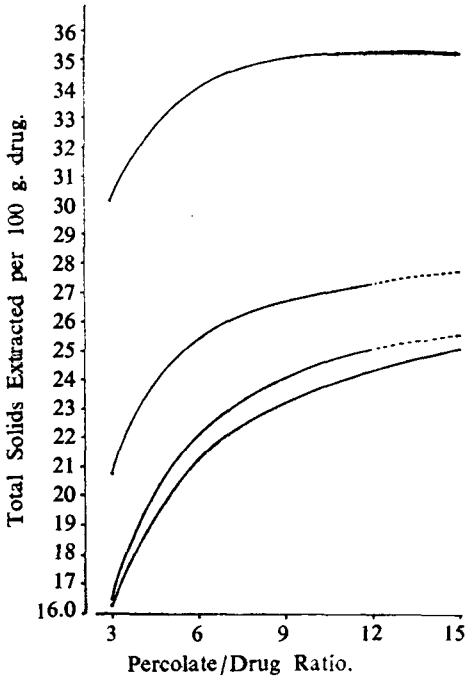

Fig. 4. Extraction of soluble extractive of senna pod, in coarse powder, by percolation with aqueous dilutions of organic solvents. Topmost curve, water; 2nd, 70 per cent. methyl alcohol; 3rd, 70 per cent. acetone; lowest, 70 per cent. ethyl alcohol.

The percolates previously made with 70 per cent. dilutions of acetone, ethyl alcohol and methyl alcohol were evaporated under reduced pressure at as low temperatures as possible. The soft extracts so prepared were assayed, and the proportion of glycosides to total solids calculated and compared with those from the corresponding percolates. Only when the temperature of evaporation did not exceed 40°C. did the values indicate that no loss of activity had occurred. For example, the 70 per cent. methyl alcohol percolate contained 101 mg. of glycosides per g. total solids; the soft extract prepared from it contained 61 mg. of glycosides per g. extract and 100 mg. of glycosides per g. total solids. This indicated that there had been a complete survival of the glycosides and the product represented an extract containing all the activity of the pod.

Discussion

Two considerations must be borne in mind when selecting a suitable method for the extraction of a drug, namely, (a) the proportion of active principles extracted and (b) the volume of solvent required. Since the active principles of senna are thermolabile, as has been shown by this investigation, it is essential that the solvent used should extract most of the activity in a reasonably small volume, in order to minimise losses during evaporation. A review of the results recorded in Tables V, VI and VIII shows that percolation is the most efficient method of extraction; with hot infusion (Table V) a drug: solvent ratio of 1 : 10 extracts only approximately 56 per cent. of the available glycosides; with triple maceration (Table VI) a ratio of 1 : 7 extracts 64 per cent., whereas with percolation (Table VIII) a ratio of 1 : 6 extracts 73 per cent. Furthermore, percolation is the most convenient process for dealing with large quantities of crude drug. The large volume of water needed for the extraction of the active principles is an outstanding feature and renders the concentrating of the extractive more difficult and costly.

In addition to losses attributed to incomplete extraction, evaporation of aqueous extractives at a temperature not exceeding 60°C. could be responsible for a further 20 to 30 per cent. loss in activity (Table IX). Studies on the effect of heat on percolates have shown that the glycosides are more sensitive to heat (especially in the alkaline *p*H range) than has been generally believed, and that losses in activity during concentration of extractives will be less the lower the temperature and shorter the time of evaporation. However, by adhering rigidly to the pharmacopœial instructions for extraction and concentration, the potency of liquid extract of senna is not likely to be more than about 50 per cent. of that of the pod; extraction by the more efficient general percolation process would increase this proportion to about 60 per cent. This appears to be the maximum which could be present in the concentrated products of commerce which have been made using water as solvent. Analyses of such extracts (Table II) indicate that less than 10 per cent. of the glycosidal content of the drug is present. These facts suggest that there may be considerable loss on storage, and it is proposed to investigate this matter in greater detail later. It is interesting to record that fresh infusion of senna B.P.C. is the most active and reliable of the official galenicals.

The amount of active principles present in extracts could be increased if evaporation were conducted at temperatures lower than 60°C. and could approach a maximum of approximately 75 per cent. of that of the pod by concentrating at a temperature which does not exceed 40°C., but such a low temperature may not be practicable on a manufacturing scale.

The use of dilutions of organic solvents is attractive because they can dissolve a higher proportion of glycosides in smaller volumes; furthermore, since the solutions can be concentrated at lower temperatures than can aqueous solutions, there will be smaller losses of activity. How-

ever, before organic solvents can be recommended for the preparation of pharmaceutical extracts, the question of their possible toxicity, and whether they extract undesirable materials, must first be considered. If the last traces of the solvent can be removed completely from the extract, and provided that the cost of the process is economic, then solvents which are more efficient than water have obvious advantages.

SUMMARY

1. The glycosidal content (as sennosides A + B) of a number of commercial samples of senna pod has been determined by chemical assay, and it has been shown that the usual criteria, appearance and water-soluble extractive, are not sufficiently reliable as methods of evaluation.

2. Alexandrian pod (*Cassia acutifolia,* Delile) contains a higher proportion of glycosides and water-soluble extractive than Tinnevelly (*C. angustifolia,* Vahl), the averages and ranges being 2·37 to **3·22** to 4·34 per cent. and 1·22 to **1·96** to 2·78 per cent. of glycosides, and 25·8 to **28·3** to 31·2 per cent. and 20·7 to **23·0** to 25.5 per cent. of water-soluble extractives, respectively.

3. The glycosidal content of a number of commercial samples of liquid extract of senna B.P. has been determined; the extracts were found to contain not more than 1/10 of the activity of the pod. Samples of concentrated infusion of senna B.P. contained not more than 1/6 of the theoretical glycosidal content.

4. The low values, in both instances, have been traced to incomplete extraction, loss on concentration and to deterioration during storage.

5. Certain non-official preparations of senna pod were assayed biologically and their glycosidal content calculated. The proportion of the theoretical activity was higher than that in official preparations.

6. Approximately 3/4 of the active principles of the pod are extracted in a simple (1·2 per cent.) infusion. Fresh infusion of senna B.P.C. (which has a drug: menstruum ratio 1 : 10) extracts only about 1/2 of the activity.

7. The B.P. extraction processes have been investigated; the general percolation process was found to be the most efficient, about 3/4 of the activity being extracted in a drug; percolate ratio of 1 : 6.

8. The effect of heat on the glycosidal content of aqueous percolates of senna pod has been studied. Between 50° and 60°C. there was a loss of 1/3 to 1/5 of the initial activity during 8 hours heating. No loss in activity took place during concentration of a percolate at a temperature not exceeding 40°C. during 10 hours' heating.

9. The active principles are almost completely soluble in 70 per cent. acetone, 70 per cent. ethyl alcohol, and 70 per cent. methyl alcohol; less solid matter is extracted by these solvents than with water. Extracts prepared by percolation with these solvents and evaporation of the percolate below 40°C. contained all the activity of the pod. The advisability of using these solvents for the preparation of an extract of senna is discussed.

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REFERENCES

1. Fairbairn, *J. Pharm. Pharmacol.*, 1949, **1**, 683.
2. Fairburn and Michaels, *ibid.*
3. Stoll, Becker and Kussmaul, *Helv. chim. Acta*, 1949, **32**, 1892.

DISCUSSION

Part II was presented by Dr. I. Michaels and Part III by Dr. Fairbairn, and the two papers were discussed together.

PROFESSOR H. BRINDLE (Manchester) asked whether the authors had considered the possibility of spray drying their aqueous extract of senna. He thought that they could have produced a 100 per cent. extract. Blood complement, which decomposed almost completely at 40°C., had been dried almost completely without loss and, therefore, there should be no loss whatever in drying extract of senna. It would be quite possible to extract senna pods with water, spray dry to a powder and compress into a tablet.

MR. T. D. WHITTET (London) asked whether the authors had tried propylene glycol as a solvent. It was a suitable solvent for a number of galenical preparations and he had found that several substances which were virtually insoluble in water were quite soluble in solutions of propylene glycol. As it toxicity was even lower than that of glycerin, it might be a suitable solvent for this type of preparation.

DR. D. C. GARRATT (Nottingham) asked if the accuracy of the chemical assays in question (± 4 per cent.) was based on a correlation with the biological activities, because he would submit that that was not permissible in view of the small number of biological assays. If it were based on the reproducibility of the chemical assays it was a reproducibility and not an accuracy which was involved.

MR. C. J. EASTLAND (London) said that he was pleased to find that the authors confirmed the results of a similar investigation by two colleagues of his, Dr. Collier and W. Bellis, which was reported to the Brighton Conference. Like the present authors, they had found a fair degree of agreement between the chemical and biological assays; later work, however, suggested that the chemical assay was likely to give rather high results when applied to really old extracts or preparations which had been artificially aged by storage at high temperature. Perhaps the modified Kussmaul and Becker's method overcame this difficulty. If not, it would indicate that the breakdown on hydrolysis of the glycosides during prolonged storage periods was different from the breakdown caused by subjecting the extracts to high temperatures.

MR. J. L. FORSDIKE (Nottingham) asked whether the authors had considered the use of dilute alcohols in the preliminary extraction of the drug for the assay. When senna pods were extracted with 45 per cent. alcohol and the extracts assayed, one would get a considerably higher result than if one extracted the same pods with water. He had extracted two samples of senna pod exactly as in Part II of the paper and also by using 70 per cent. and 45 per cent. alcohol, the filtrate in each case being assayed as described by the authors. The 70 per cent. alcohol gave a 5 to 10 per cent. higher red colour intensity than the aqueous alkali extraction and 45 per cent. alcohol gave as much as 20 per cent. more colour, which indicated a higher combined anthraquinone content in the drug and seemed to suggest that the whole of the anthraquinone glycosides present was not extracted by the method described in the paper.

Three different methods of extraction had been described, one for the whole pod, one for the coarsely-powdered pod and one for the fine powder. In the first two the volume was made up to 10 g. in 500 ml., but for the fine powder it was 1 g. in 100 ml. Was there any reason for this difference, or any objection to powdering the whole or partially-crushed material finely before use? The authors had heated the alkaline solution for 4 to 5 minutes. He thought it necessary to control that time of heating very accurately to 4 minutes, as after this time the colour decreased. It was also essential to examine the colour immediately, and this was not specified in the paper, because the red colour faded within a period of an hour or so. In Part III of the paper the authors had examined the potency of commercial samples of liquid extract of senna B.P. by chemical assay and found only 1 to 8 per cent. of the theoretical sennoside content (Table II) but in non-official preparations of senna pod, assayed biologically (Table III), they had found 30 to 40 per cent. and in one case over 100 per cent. Were the B.P. extracts also assayed biologically, as the results might prove to be of some significance. Tables VI, VII and VIII (Part III) showed that the authors had found only a proportion of the expected amount of glycosides to be extracted by the three methods. Had they determined whether the missing glycosides were left in the marc or whether they had been destroyed in the process of extraction?

MR. H. DEANE (London) said that Dr. Fairbairn had referred to certain proprietary preparations as being highly active, but was he sure that they did not contain other purgatives than senna, as that was quite a likely possibility? He thought that they should investigate the deposit which forms when these preparations are stored. The authors had not given any details about the colour standard. He presumed that the pure glycosides were not commercially available and that it was necessary for the analyst to prepare them and to make his own standards with the colorimeter which he was using.

MR. G. R. A. SHORT (London) commented on the absence of any correlation between the appearance of the pods and their activity. Pods

used in manufacturing often gave a highly active product in spite of their poor condition. He wondered whether the degree of ripeness affected the activity of the pods. He suggested that a creeping film evaporator would be very suitable for the evaporation of the senna extracts. The period of heating would only be a few seconds, and, even when the concentrated liquid was subsequently evaporated to dryness, it would not be necessary to heat for 10 hours as suggested by the authors. There was no need to fear the toxic effects of the solvents used, as manufacturers made sure that they were entirely removed from the final product.

DR. G. H. MACMORRAN (Edinburgh) asked why the authors had adjusted the infusion to *p*H 3. Straub and Gebhardt had reported that one of the glycosides was hydrolysed by acids during extraction. This being so, the aglycone would be extracted by the organic solvent and the final figure in the chemical assay would be smaller than the correct figure. He thought that the similarity between the chemical and biological assays was very strange as biological methods were generally considered to have a wide variability. It might be that a low figure was being shown in each, due, in the case of the chemical assay, to the hydrolysis of one of the glycosides.

DR. G. E. FOSTER (Dartford) asked which filter had been used with the Spekker absorptionmeter. DR. MICHAELS replied that it was the 604.

DR. F. J. DYER (London) asked whether the authors had tried the use of 60 per cent. alcohol plus glycerin, as was done in the extraction of the digitalis glycosides by one or two of the older commercial methods.

DR. I. MICHAELS, in reply, said that they had no spray drier and they had read that spray drying resulted in the extracts being somewhat hygroscopic. Propylene glycol was not used as its boiling-point was rather high and the glycosides were thermolabile. They agreed with Dr. Garratt that they should have referred to limits of reproducibility rather than limits of accuracy. The assays had been done a number of times on the powders and on the whole pod, on different days, as the process was rather prolonged at first, and they statistically analysed the results. One person was able to do two assays in a working day. The results were often identical and never varied by more than about 5 per cent. Mr. Eastland had pointed out that an old extract frequently gave high chemical results. The extracts which they had bought must have been several months old, and they were stored in the laboratory for six months before being assayed. These samples gave very low results. The effect of ripeness on the activity of samples of pods had not been investigated, but they would bear this in mind. The reason for the period of 10 hours being required for the evaporation of one extract was that they were careful to keep the temperature of the water-bath below 50°C. Although this was a long time, there was small likelihood of much decomposition. They had thought of using the creeping film evaporator, but had then given up investigating aqueous extracts

as they were not satisfied that they had extracted all the active principles, and their goal was to produce an extract which represented 100 per cent. of the activity of the product. The creeping film evaporator would have been useful for producing a 75 per cent. active preparation.

DR. J. W. FAIRBAIRN said that he had drawn the attention of Dr. Collier to the fact that the chemical assay process estimated only glycosides, and therefore he could not understand why with an old preparation, which had a low biological activity, a high result was obtained with the chemical assay. Mr. Eastland's suggestion that there might be different types of breakdown on hydrolysis was worth thinking about. With regard to Mr. Forsdike's remarks, it was important to remember that there were other glycosides and other anthraquinone compounds in senna which were not assayed by the Kussmaul and Becker process. Their results, however, had shown that, while the sennosides might not account for the total activity, they very nearly did so. They had been very surprised by the close agreement between the biological and chemical assay results. The biological process was accurate only within 15 to 20 per cent. He expected that future results would differ more widely. The exact composition of the proprietary preparations tested had not been known. There might have been some synergistic effect of other ingredients, and they had had to use the biological rather than the chemical assay. The pure glycosides had been obtained from Professor A. Stoll. If one had to make them, the instructions were given in the literature for doing so, but it might entail three months' work. In his opinion, the easily hydrolysable glycoside of Straub and Gebhardt did not exist.