VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART VI. THE INSTABILITY OF LIQUID PREPARATIONS OF SENNA DURING STORAGE

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INTRODUCTION

IN an earlier paper in this series Fairbairn and Michaels[1] showed that if the directions for the preparation of liquid extract of senna B.P. 1948 are followed, only about two-thirds of the sennosides are extracted from the pod in the combined macerates. They also showed that there would be a further loss of about 20 to 30 per cent. in the subsequent process of concentration by evaporation. Commercial samples of the extract they examined, however, contained only about 1 to 10 per cent. of the glycosides of the pod and they concluded that these low glycosidal contents were not only due to the factors already mentioned, but were also possibly due to the effect of storage. This conclusion is confirmed by results reported by workers in America, especially Geiger[2], Wiebelhaus and Lee[3], and Hazleton and Talbert[4,5], who used biological methods of assay. These workers found that the fluid extract of senna U.S.P. XII gradually lost potency on storage and the commercial samples only contained about 15 to 25 per cent. of the supposed activity[2]. These unsatisfactory results partly account for the omission of senna and its preparations from the U.S.P. XIV[6].

The syrup and infusion of senna were found to be more stable than the fluid extract[5], while the crude drug itself showed no appreciable loss of activity after storage for 5 years or more[7,8].

It should be pointed out that the stability tests carried out by the American workers were based on biological methods whose accuracy has been criticised by Lou[9]. However, the results agree approximately with those already described by Fairbairn and Michaels, who used chemical methods of assay. We decided therefore to re-investigate the effect of storage on extracts of senna, using a chemical method of assay whenever possible. To do this it was necessary to determine what components were responsible for the purgative action of the extract and then to determine the effect of different conditions of storage on these components. As a result of this investigation we have shown that sennosides A and B are wholly responsible for the activity of the extract and that these glycosides are fairly rapidly decomposed in warm conditions, by the presence of ethanol, and by an alkaline *p*H. There was also some evidence that hydrolysis of the glycosides was partly due to enzyme action.

EXPERIMENTAL

Preparation of a Potent Liquid Extract A

To 500 g. of Alexandrian senna pod (cut into small pieces) 4 l. of distilled water at 60° C. was added and the mixture allowed to cool and kept at room temperature for 16 hours. The liquid was strained and evaporated to a syrupy consistence under reduced pressure at a temperature not exceeding 40° C. The marc was macerated once more in the same manner for 3 hours, the macerate concentrated as before and the two concentrated fractions were mixed. Total time of distillation was 6 hours. The extract was heated at 85° C. for 5 minutes, cooled rapidly under the tap to room temperature, kept for 48 hours to allow proteinous matter to settle, then filtered. The volume was about 900 ml. and chemical assay showed that it contained 1·06 per cent. w/v of sennosides A + B and represented about 50 per cent. of the glycosides originally present in the pod.

Active Constituents of the Liquid Extract

The activity of the liquid extract was compared biologically with that of sennosides A and B by the method of Lou[9]. The results are recorded in Table I.

TABLE I

COMPARISON OF THE BIOLOGICAL ACTIVITIES OF LIQUID EXTRACT OF SENNA A AND SENNOSIDES A AND B

Material	Weight of sennosides in average of the two dose-levels mg.	Biological activity sennosides A and B = 100
1. Sennosides A and B (equal quantities) ..	0·90	100
2. Liquid extract of senna, A	0·90	(i) 95, (ii) 97, (iii) 111 } 101

The results show that sennosides A and B account for the entire activity of the extract. Such a fact is of great importance as the chemical assay of the extract will be a direct measurement of its biological activity. This is in contrast with the crude drug, where the sennosides only represent about two-thirds of the activity[12].

THE EFFECT OF VARIOUS CONDITIONS OF STORAGE

The extract was stored for different periods under different conditions. In each of the following experiments, 40 ml. of the extract was diluted with 10 ml. of distilled water or absolute ethanol as specified and the whole placed in dry 60-ml. bottles or in 50-ml. ampoules. All the bottles were glass-stoppered and tightly closed except in the experiment to see the effect of air. 0·1 g. of chlorocresol was dissolved in each 50 ml. of water-diluted extract to prevent formation of mould (except in the autoclaving experiment). The *p*H of the extract, unless specified, was 5·04. In order to simplify the recording of our numerous results we have grouped them into 5 diagrams and 3 tables, each set of which is discussed below.

1. *Effect of ethanol, oxygen and light* (see Figure 1 and Table II). The results show that light has little deleterious effect and that exposure to air has a slightly more deleterious effect though storage with an antioxidant does not lead to any increase in stability when stoppered bottles are used. The presence of ethanol, on the other hand, leads to a marked loss in activity and the next set of experiments confirmed this observation (see Figure 2 and Table II). This is an important result as dilute ethanol is frequently used as a preservative for liquid preparations of senna; it also probably explains why previous workers found that the syrup and infusion, which contain little or no ethanol, were more stable than the fluid extract which contains about 25 per cent. of ethanol.

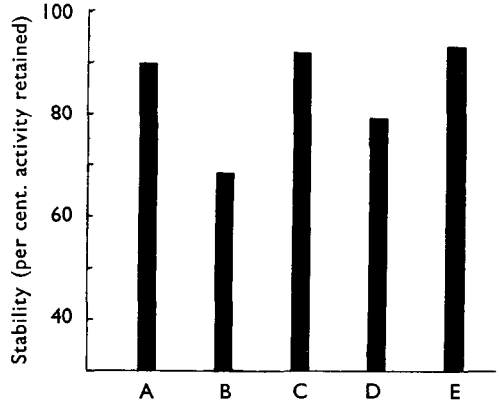

FIG. 1. Effect of storage at room temperature for 28 weeks under various conditions.

A = Control.
B = With 20 per cent. of ethanol.
C = With 0·2 per cent. of sodium metabisulphite.
D = Exposed to air.
E = Protected from light.

TABLE II

EFFECT OF ETHANOL, OXYGEN, LIGHT AND TEMPERATURE ON THE STABILITY OF SENNA EXTRACTS DURING STORAGE

Material	Temperature	Age weeks	Content of sennosides A + B (mg./ml.)	Stability (sennosides retained) per cent.
1. Fresh extract A	—	—	8·5	—
2. Control sample A	Room	28	7·6	89
3. Control A, plus 20 per cent. of ethanol	Room	28	5·8	68
4. Control A + deoxidant (0·2 per cent. of sodium metabisulphite)	Room	28	7·8	92
5. Control A, exposed to air*	Room	28	6·7	79
6. Control A, protected from light	Room	28	7·5	88
7. Control A	37° C.	14	3·8	45
8. Control A plus 20 per cent. of ethanol	37° C.	14	3·0	35
9. Fresh extract B (after heating at 80° C. for 3 minutes)	—	—	11·6	—
10. Control sample B	Room	14	10·9	94
11. Control sample B	37° C.	10	7·1	61
12. Pure sennosides in water	Room	14	—	100
13. Pure sennosides in water	37° C.	10	—	95

* The extract was made up to volume after storage before being assayed.

2. *Effect of temperature* (see Figure 2 and Table II). The results show that storage at elevated temperature leads to rapid loss of activity; thus, while storage at room temperature for 14 weeks led to a loss of only 6 per cent., storage at 37° C. for 10 weeks led to 40 per cent., and at 37° C. for 14 weeks led to 55 per cent. loss. On the other hand, storage of the aqueous solution of the pure glycosides at 37° C. for 10 weeks resulted in a negligible loss of activity (5 per cent.), thus indicating that enzymes in the extracts may be accelerating the loss.

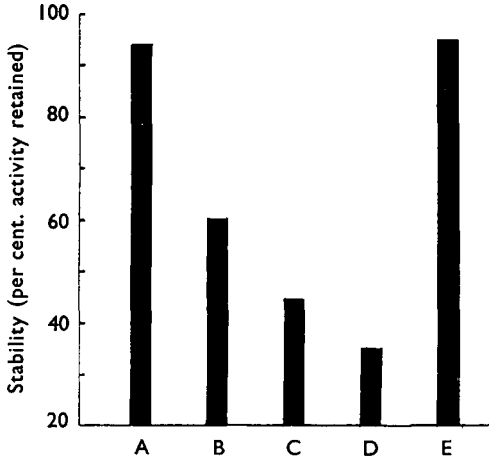

FIG. 2. Effect of temperature during storage.
A = Extract B at room temperature, 14 weeks.
B = Extract B at 37° C., for 10 weeks.
C = Extract A at 37° C., 14 weeks.
D = Extract A plus 20 per cent. of ethanol at 37° C., 14 weeks.
E = Pure sennosides in water at 37° C., 10 weeks.

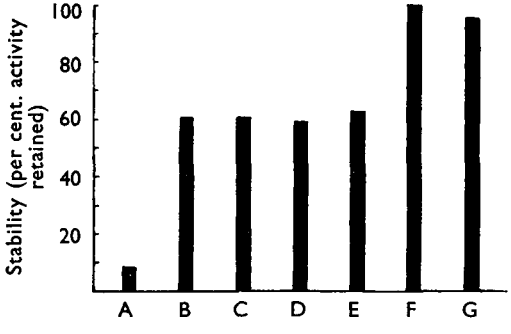

FIG. 3. Effect of preliminary heat treatment. All samples stored at 37° C. for 10 weeks, except F, which was stored for 28 weeks at room temperature.
A = Unheated.
B = Heated 3 minutes at 80° C.
C = Heated 5 minutes at 85° C.
D = Heated 10 minutes at 85° C.
E = Heated 20 minutes at 85° C.
F = Heated 30 minutes at 110° C.
G = Pure sennosides in water (see Table II).

3. *Effect of preliminary heat treatment* (see Figure 3 and Table III). This series of experiments was designed to obtain further evidence for the presence of enzymes, and if possible to prevent their deleterious effects. A further batch of concentrated extract (B) was prepared in exactly the same manner as the first one (A) except for the omission of the preliminary heat treatment (85° C. for 5 minutes); thus ensuring no destruction of enzymes. This new batch was necessary as a control and was used for this series of experiments, except in the autoclaving experiment, where extract A was used.

The results show that without a preliminary heat treatment there is a large loss of activity on subsequent storage. This loss was partly prevented by the short preliminary heat treatment at 80° C.

specified in the B.P. 1948, and it is interesting to note that increasing the temperature to 85° C. and the time of this treatment up to 20 minutes does not lead to any greater stabilisation. The loss of activity on storage was completely prevented by autoclaving; however, the process of autoclaving produced an immediate loss of 65 per cent. of activity so that this method of stabilisation is impracticable.

A possible explanation of these results is that the extract contains two enzyme systems, one readily destroyed by heating at 80° C. and the other requiring a temperature of about 110° C. to destroy it; it will be noted that the autoclaved extract has a stability equal to that of an aqueous solution of the pure glycosides. In view of the conclusions we ultimately arrived at, and which we discuss at the end of this paper, we decided not to pursue the question of possible enzyme systems any further.

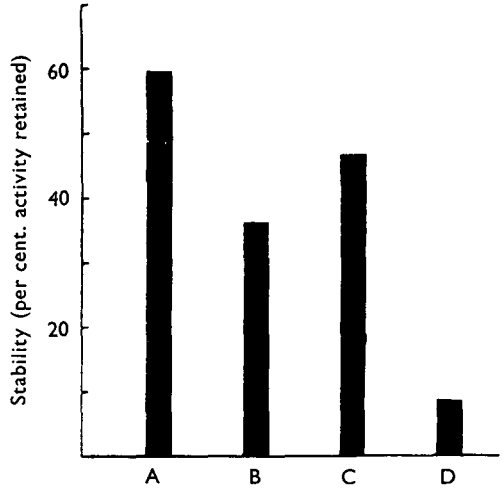

FIG. 4. Effect of preservatives. All samples stored at 37° C. for 10 weeks.

A = Chlorocresol, 0·2 per cent. w/v.
B = Ethanol, 20 per cent. v/v.
C = Ethanol, 20 per cent. and chlorocresol, 0·2 per cent.
D = Chloroform, 0·2 per cent. v/v.

4. *Effect of preservatives* (see Figure 4 and Table III). This set of experiments represented a further attempt to depress the activity of the postulated enzymes and accordingly extract B (prepared without any preliminary heat treatment) was used. The results show that chlorocresol is the best depressant, ethanol is less effective and chloroform has little depressant effect.

5. *Effect of* p*H*. Samples of extract A and extract B (after preliminary heat treatment at 85° C. for 3 to 5 minutes) and preserved with 0·2 per cent. of chlorocresol, were adjusted to various *p*H values and stored both at room temperature and at 37° C. The results of this series of experiments are shown graphically in Figure 5. These results show that at *p*H values above 5 there is a fairly rapid loss in stability, whereas at values below 5 there seems to be little increase in stability. It is interesting to note that the natural *p*H value of aqueous extracts of senna is about 5. There is some evidence that at *p*H 3·5 there is a further increase in stability and it is interesting to note that an aqueous solution of the sennosides has *p*H 3·6.

However, at these low *p*H values there occurs a considerable amount of precipitation during storage. The sennosides are sparingly soluble in

cold water but occur in the crude drug mostly as readily soluble salts, in which form they will pass into the extracts. Acidification of these extracts will result in the formation of "free" sennosides which would be precipitated; hence it seems obvious that the precipitates already reported to occur at low *p*H values would contain sennosides. Two series of experiments were carried out at room temperature and at 37° C. to confirm this conclusion and the results are shown in Tables IV and V.

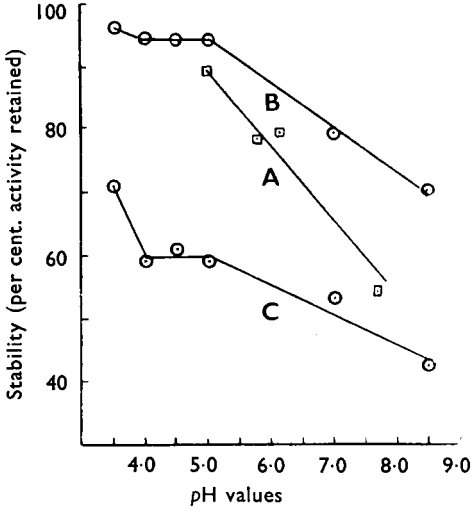

FIG. 5. Effect of *p*H.

A = Extract A, stored at room temperature for 28 weeks.
B = Extract B, heated at 85° C. for 5 minutes, stored at room temperature for 14 weeks.
C = Extract B, as above, stored at 37° C. for 10 weeks.

It will be seen that at *p*H values above 4·5 no precipitation of the glycosides occurs; at lower *p*H values precipitation increases, being about 70 per cent. at *p*H 3·5.

DISCUSSION

Some of the facts reported in this paper confirm information reported by other workers. Thus Stoll, Kussmaul and Becker[10] state that the sennosides are relatively unstable in alkaline solution and Fairbairn and Michaels[1] found that an increase in *p*H led to an increase in glycosidal loss during the evaporation of senna extracts. Hazleton and Talbert[5] and Fairbairn and Michaels[1] have shown that high temperature causes comparatively rapid hydrolysis of the glycosides. Stoll, Kussmaul and Becker[10] state that the sennosides can be decomposed by alcoholysis, which may account for the deleterious effect of ethanol in senna extracts. The effect of enzymes was studied by Wiebelhaus and Lee[3], who reported that an extract made from the autoclaved drug remained stable indefinitely, though Vincent, Walden and Lee[11] showed that autoclaving senna led to an immediate loss of about 85 per cent. of the activity.

The most obvious result of this present work has been to demonstrate that the requirements necessary to produce optimum stability prevent, at the same time, production of a suitable extract. Thus the deleterious effects of the postulated enzyme system can be readily prevented by autoclaving the extracts, but this leads to an immediate loss of two-thirds of the activity. Again, the optimum *p*H for stability is about 3 to 4, but at this value there is considerable precipitation of the glycosides during storage. Thirdly, ethanol, which is commonly used as a preservative in galenical preparations has been shown itself to have a marked

deleterious effect on the glycoside; chlorocresol, on the other hand, has no such deleterious effect but may not be a suitable preservative to add to a preparation which is to be taken in comparatively large doses. Finally, it has long been known that senna extracts produce deposits

TABLE III

EFFECT OF INITIAL HEAT TREATMENT AND PRESERVATIVES ON THE STABILITY OF SENNA EXTRACTS DURING STORAGE

Material	Initial heat treatment	Age and storage temperature	Content of sennosides A + B (mg./ml.)	Stability (sennosides retained) per cent.
1. Fresh extract B	—	—	11·6	—
2. Extract B after storage	unheated	10 weeks at 37° C.	1·0	9
3. „ „ „ „	3 minutes at 80° C.	„	7·1	61
4. „ „ „ „	5 minutes at 85° C.	„	7·0	60
5. „ „ „ „	10 minutes at 85° C.	„	6·9	59
6. „ „ „ „	20 minutes at 85° C.	„	7·3	63
7. Extract B plus 0·2 per cent. chlorocresol	unheated	„	6·9	59
8. Extract B plus 20 per cent. ethanol	unheated	„	4·1	35
9. Extract B plus 20 per cent. ethanol and 0·2 per cent. chlorocresol	unheated	„	5·4	47
10. Extract B plus 0·2 per cent. chloroform	unheated	„	1·0	9
11. Fresh extract A	—	—	8·5	—
12. Extract A	autoclaved	1 day at room temperature	3·0	—
13. „ A	„	28 weeks at room temperature	3·0	100

TABLE IV

EFFECT OF *p*H ON THE PRECIPITATION OF THE SENNOSIDES IN LIQUID EXTRACT OF SENNA DURING STORAGE

(Stored at room temperature)

Material	*p*H	Age weeks	Content of sennosides as (A + B) mg./ml.	
			Whole extract	Supernatant liquid
1. Fresh extract, B*	5·0	—	11·6	—
2. Same after storage	3·5	14	11·1	3·7
3. „ „ „	4·0	„	10·9	6·5
4. „ „ „	4·5	„	10·9	10·8
5. „ „ „	5·0	„	10·9	10·9
6. „ „ „	7·0	„	9·2	9·2
7. „ „ „	8·5	„	8 1	8·1

* The fresh extract showed no deposit.

on storage; we have shown that at *p*H values above 5 these deposits would contain little glycoside but nevertheless they are unsightly. We have therefore concluded that liquid preparations of senna, containing water or ethanol as vehicle, are not suitable galenicals, not only because of their instability but because if water is used as the solvent, it is impossible to extract and retain initially more than 50 to 60 per cent. of the glycosides from the crude drug[1].

Fairbairn and Michaels[1] have already shown that it is possible to extract conveniently, practically all the sennosides from senna pod if

TABLE V

EFFECT OF *p*H ON THE PRECIPITATION OF THE SENNOSIDES IN LIQUID EXTRACT OF SENNA DURING STORAGE AT 37° C.

Material	*p*H	Age weeks	Content of sennosides as (A + B) mg./ml.	
			Whole extract	Supernatant liquid
1. Fresh extract, B*	5·0	—	11·6	—
2. Same after storage	3·5	10	8·2	2·5
3. „ „ „	4·0	„	6·9	4·0
4. „ „ „	4·5	„	7·1	6·9
5. „ „ „	5·0	„	6·9	6·9
6. „ „ „	7·0	„	6·2	6·1
7. „ „ „	8·5	„	4·9	4·9

* The fresh extract showed no deposit.

ethanol (70 per cent.) is used as a solvent, and since this solvent can be evaporated off at a lower temperature than water there will be little loss of glycosides during concentration. Accordingly, we prepared an extract by percolating 1 part of powdered senna pod with 15 parts of ethanol (70 per cent.). The percolate was evaporated to dryness under reduced pressure at a temperature not exceeding 30° C. The extract was then dried in a vacuum desiccator until of constant weight and was analysed chemically and pharmacologically. The chemical assays showed that the sennosides of the pod had been completely extracted and retained. The pharmacological assay showed further that the "non-anthracene" active fraction was also in the final extract.

We have already shown[12] that two-thirds of the activity of the crude drug is due to its sennoside content and that the remaining third of the activity is due to this unknown "non-anthracene" factor. It can be seen from Table I that the activity of extract A, which was made more or less according to the method described in the B.P. 1948, is entirely accounted for by its sennoside content. This means that the "non-anthracene" factor has been destroyed during preparation and gives further cause for rejecting the present methods of making liquid preparations of senna. At most only 50 per cent. of the sennosides will be retained in the finished preparation and as these sennosides only represent two-thirds of the

activity of the crude drug, the extract can never contain more than one-third of the original activity. On the other hand, using ethanol (70 per cent.) all the glycosides and the "non-anthracene" factor are extracted and retained so that the finished product contains all the original activity. Furthermore, since it contains very little water, it would be expected to retain its activity well during storage, as is true for the crude drug[7,8].

The ethanol (70 per cent.) extract was brown with a slightly acrid taste; it is only slightly hygroscopic but dissolves readily in water, the resulting solution having a *p*H of about 5. It is hoped to investigate its suitability as a basis for such pharmaceutical preparations as granules, tablets, etc.

Summary

1. It has been shown that, in contrast to the crude drug, the purgative activity of a fluid extract of senna pod is entirely due to its content of anthracene glycosides (the fluid extract therefore does not contain the "non-anthracene" active fraction[12] of the crude drug).

2. Chemical assays for glycosidal content have therefore been used to determine the effect of various conditions of storage on the stability of fluid extracts of senna.

3. The most important factor is the preliminary heat treatment; without it there is a very marked loss in activity during subsequent storage. However, preliminary heating at 80° C. for 3 minutes, as recommended in the B.P. 1948, does not prevent considerable loss of activity during storage. This loss can be entirely prevented by preliminary autoclaving, but, as this process leads to an immediate destruction of two-thirds of the glycosides, it is impracticable.

4. Storage at elevated temperature (e.g., 37° C.) leads to rapid loss.

5. The presence of ethanol (as a preservative) has a marked deleterious effect on the activity. Chlorocresol has no such deleterious effect but it is probably an unsuitable preservative for this type of galenical.

6. *p*H values below 6 or 7 lead to rapid loss in activity during storage; maximum stability seems to be attained at *p*H 3 to 4 but at this *p*H value the sennosides precipitate rather rapidly.

7. Exposure to light and air have comparatively little effect on stability.

8. These results are discussed and it is concluded that liquid preparations of senna containing water or ethanol are unsatisfactory galenicals.

9. An ethanol (70 per cent.) extract of senna pod was prepared and carefully evaporated to dryness. The dry extract not only contained all the glycosides from the pod but also the "non-anthracene" active fraction. Since the moisture content is low it would be expected to retain its activity well during storage, as has been reported for the air dried drug[7,8].

10. It is hoped to use this dry extract as the basis for the manufacture of suitable pharmaceutical preparations.

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work, and Mr. J. H. Davey for assistance with the biological assays. Some of this work has been included by one of us (M.R.I.S.) as part of a Thesis for the Ph.D. degree of the University of London.

References

1. Fairbairn and Michaels, *J. Pharm. Pharmacol.*, 1950, **2,** 813.
2. Geiger, *J. Amer. pharm. Ass., Sci. Ed.*, 1940, **29,** 148.
3. Wiebelhaus and Lee, *ibid.*, 1943, **32,** 165.
4. Hazleton and Talbert, *ibid.*, 1944, **33,** 170.
5. Hazleton and Talbert, *ibid.*, 1945, **34,** 260.
6. Private communication.
7. Grote and Woods, *J. Amer. pharm. Ass., Sci. Ed.*, 1944, **33,** 266.
8. Grote and Woods, *ibid.*, 1951, **40,** 52.
9. Lou, *J. Pharm. Pharmacol.*, 1949, **1,** 673.
10. Stoll, Kussmaul and Becker, *Helv. chim. Acta*, 1949, **32,** 1892.
11. Vincent, Walden and Lee, *J. Amer. pharm. Ass., Sci. Ed.*, 1945, **34,** 211.
12. Fairbairn and Saleh, *J. Pharm. Pharmacol.*, 1951, **3,** 918.

Correction.

A TASTELESS DERIVATIVE OF CHLORAMPHENICOL

By E. P. Taylor.

This Journal, 1953, **5**, 254.

On page 255, heading of Table I,

for infecting *read* infective.

On page 256, reference 1 should read:—

Belgian Patent 503,675. Parke, Davis and Co.