SPECTROPHOTOMETRIC ESTIMATION OF SENNOSIDES AND RHEIN GLYCOSIDES IN SENNA AND ITS PREPARATIONS

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ABSTRACT.—A spectrophotometric method is presented for assaying Senna and its preparations, for both total sennosides and total rhein glycosides content. The method ensures complete elimination of other minor non-carboxylic anthracene derivatives, as well as flavonoidal contaminants. The proposed method quantitates the actual total sennosides content, through the elimination of these contaminants, and through correction for the interference due to the coexistence of rhein with sennidins, in the final determinative step. This would eliminate false high figures for total sennosides by earlier procedures and reflects, perspectively, the actual potency of the assayed samples.

Senna leaf and fruit, as well as their preparations, are valuable drugs that have gained prominence and historical reputation as purgatives. The importance of these drugs is best demonstrated by their inclusion in most current pharmacopoeias (1).

Development of a satisfactory chemical assay for the drug has long been hampered by previous lack of knowledge of the precise active principles of the drug (2).

The P.P.C. method of assay of senna leaf and the B.P. method of assay of senna tablets, like most reported colorimetric methods, are based on the same principle, i.e., aqueous extraction, removal of free anthraquinone genins, oxidative cleavage of dianthrones and acid hydrolysis to give monomeric anthraquinone genins that are then determined colorimetrically by Borntragers' reaction; the total glycosides are then calculated in terms of sennoside B (3, 4).

Minor differences in other reported methods are centered in the initial extraction step, in the reagents utilized in oxidative cleavage and hydrolysis and, in the solvent recommended for extraction of the genins (5–8).

Clues to the precise constituents of the drug were provided by Friedrich and Baier (9), who reviewed results of previous investigations and studied comprehensively the nature of these constituents. Besides the dianthrone glycosides (sennosides), senna contains rhein-8-glucoside, rhein-8-diglucoside, rhein-anthrone-8-glucoside, aloe-emodin dianthrone diglucoside, and aloe-emodin glucoside (9, 10). Aloe-emodin anthrone diglucoside and palmidin A were also reported (2, 9).

It was due to the research of Fairbairn and co-workers (11, 12) that activity was ascribed mainly to the dimeric glycosides. This urged other investigators to seek new methods of assay for the quantitation of these dimers. Thus, Auterhoff and Kinsky (13) developed a spectrophotometric method for the estimation of sennosides that was later modified by Brendel and Schneider (10, 14, 15). The modifications by Brendel et al. were aimed to eliminate false high results for sennosides by the former assay procedure.

Our spectral studies, however, proved the procedure used by Brendel *et al.* still gave figures for sennosides much higher than true values. So, we are reporting herein a method for the estimation of total sennosides and total rhein glycosides. The proposed method estimates true dimeric content through the consideration of the co-existing carboxylic monomers, mainly rhein glycosides.

MATERIAL AND METHODS

PLANT MATERIAL.—Different commercial samples of the leaf and fruit of Cassia senna and the fruit of *C. angustifolia* were used.

REFERENCE MATERIALS.—Reference samples of individual sennoside A and sennoside B were kindly provided[1] in very pure form, as revealed by tlc scrutiny conducted in different solvent systems. Thus, reference solutions of sennidin A and sennidin B were obtained by simple acid hydrolysis of the corresponding glycoside and subsequent recovery of the liberated genin.

Out of the available rhein samples, one[2] was shown by tlc to be pure compound, and gave a uv-spectrum[3] (MeOH) showing maxima at 228–9, 258, 431–5 nm, characteristic of rhein (16, 17).

Two samples of aloe-emodin were available[4]. One revealed a minor reddish contaminant of higher R_f value, while the other was chromatographically pure. The uv-spectrum[3] (EtOH) of the latter showed the characteristic maxima at 225, 254, 276, 287, 430 and 457 nm (16).

PREPARATION OF REFERENCE SENNIDIN A AND SENNIDIN B.—To 4.1 mg of sennoside A (or sennoside B), in a boiling flask, 20 ml of water and 8 ml of 25% HCl were added, and the mixture was refluxed in a boiling water bath for 20 minutes. After cooling, the mixture was quantitatively transferred into a separator. The boiling flask was washed first with 5 ml of water then with 20 ml of ethyl acetate. In each case the washings were quantitatively transferred to the separator. The mixture was shaken vigorously and, after phase separation, the ethyl acetate phase was saved. The aqueous solution was reextracted with further fractions of 15 and 10 ml of ethyl acetate. The combined ethyl acetate solution was washed with 10 ml of water, then transferred into a 50 ml volumetric flask. The original aqueous solution and the aqueous washings were extracted with the same 5 ml of ethyl acetate, and the latter used in completing the volume of the solution in the volumetric flask. As 4.1 mg of sennoside would give by simple acid hydrolysis 2.55 mg of sennidin, an additional 1 ml of ethyl acetate was added, so as to get 51 ml of a solution containing 50 ug of sennidin A (or sennidin B) per one ml.

STANDARD SOLUTIONS.—For recording the uv-spectra and the determination of absorptivity, ethyl acetate solutions containing 20 ug per ml, were prepared for each of sennidin A, sennidin B, aloe-emodin and rhein.

THIN-LAYER CHROMATOGRAPHY.—Tlc was performed on silica G chromatoplates with benzene-acetic acid (4:1) as solvent system. Spots on the developed chromatograms were visualized first under uv light, and then by spraying with 5% methanolic protassium hydroxide solution.

ASSAY PROCEDURE (CHART 1).—The powdered drug (1 g) was extracted with 30 ml of water in a water bath at 80° for one hr with frequent agitation. After cooling, the aqueous extract was decanted and filtered into a 100 ml volumetric flask; the marc was saved in the original flask. The marc was reextracted with another portion of 30 ml of water for 30 minutes; the extract was decanted and filtered through the same filter into the volumetric flask. The marc, the flask, and the filter were washed with small successive volumes of warm water; the washings were used to complete the volume of the solution in the volumetric flask.

A portion (10 ml) of the aqueous solution was transferred to a separatory funnel, then acidified with one drop of HCl, and extracted with two successive portions of 40 and 20 ml of ether. The aqueous solution was saved in a boiling flask. The combined ether extract was washed with 2 x 5 ml fractions of water, the ether phase was discarded and the aqueous washings were added to the saved aqueous solution in the boiling flask. Eight ml of 25% HCl was added and the mixture refluxed in a boiling water bath for 20 minutes. The mixture, with any precipitate, was transferred quantitatively to a separator; 5 ml of warm water was used to transfer any retained precipitate. The mixture was then extracted with 3 x 30 ml fractions of ethyl acetate, the brown deposit (at the interphase) being taken each time with the aqueous phase, and the ethyl acetate solution was filtered into a 100 ml volumetric flask. The filter was washed with small volumes of ethyl acetate, and the filtered washings were used to complete the volume of the ethyl acetate solution.

A series of three separators was prepared: 25 ml of the ethyl acetate solution was transferred to the first, and 20 ml of pure ethyl acetate to each of the other two. The three separators were shaken, in a consecutive manner, with the same two portions of 25 and 20 ml of freshly prepared saturated sodium bicarbonate solution. The ethyl acetate solutions were discarded. The combined bicarbonate solution was acidified with 10 ml of HCl; and the mixture was shaken very gently to help complete elimination of any latent effervescence, then extracted successively with 25 and 20 ml of ethyl acetate. The combined ethyl acetate solu-

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[1]Roha Arzeneimittel GmbH & Co. KG.

[2]Rhein "Sandoz".

[3]A Pye-Unicam SP6-400 spectrophotometer was used.

[4]Provided kindly by Dr. F. Soliman (Acknowledgment).

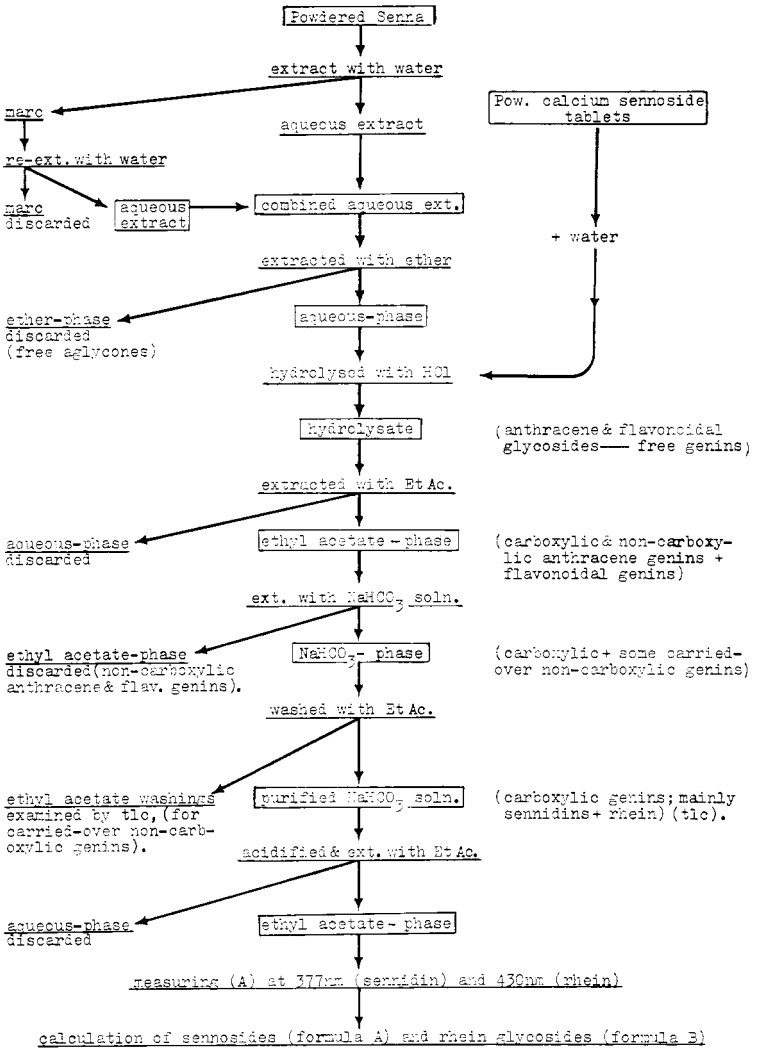

CHART I. Flow scheme for the assay of senna and calcium sennoside tablets.

tion was washed with 10 ml of water, transferred into a 50 ml volumetric flask and completed to volume. The extinction of the solution was measured against pure solvent at 377 and 430 nm, and the percentage of sennosides and total rhein glycosides was calculated, by application of formula A and formula B (below), respectively.

ASSAY OF CALCIUM SENNOSIDE TABLETS.—The tablets (20 tablets) were weighed and reduced to a very fine powder. To a weight equivalent to one tablet, 30 ml of water and 10 ml of 25% HCl were added; the assay was continued as above, starting from the statement: ". . . and the mixture refluxed in a boiling water bath, for 20 minutes.".

RESULTS AND DISCUSSION

After a review of reported methods of assay of senna, the spectrophotometric method by Brendel *et al.* (10) seemed to be the most relevant (14, 15). However, the present spectral and chromatographic studies provided evidence of a positive

error by that procedure (table 2). This is ascribed here to the following two factors:

1. The neglect of the interference of rhein with the sennidins maximum at 375 nm in the Brendel *et al.* procedure (10, 14), as well as in the former Auterhoff *et al.* procedure (13).

In this respect, we disagree with a key statement by the latter authors, that was also cited by the former authors, that is: "An artificially prepared 20 : 80 rhein to sennidin mixture, showed no significant change in maximum nor in magnitude from those of sennidins only (13, 14)."

Our spectral measurements indicated the absorptivity of rhein at 377 nm (sennidins maximum)[5] to be about 50% that of a 2 : 3 sennidin A : B mixture (table 1 and figure 1). This would obviously introduce a serious positive deviation from true sennosides content (table 2).

TABLE 1. Absorptivity values for rhein, sennidin A,B and 2:3 sennidin A: sennidin B mixture.

Compound	at 377 nm.	at 430 nm.
Sennidin A	33.0	7.75
Sennidin B	37.0	8.5
2:3 sennidin A: sennidin B mixt.	35.4	8.2
Rhein	18.5	47.5

2. The assumption, by these previous reports, that upon partitioning total genins between aqueous sodium bicarbonate and organic solvent, it is only the carboxylic genins that would pass into the aqueous bicarbonate, leaving behind in the organic phase a solution free from these, but containing all other constituents (10, 13, 14).

We found the sodium bicarbonate solution, in Brendels' procedure (10, 14), to carry over significant amounts of non-carboyxlic genins. The contribution of these to the total absorbance at 377 nm, was found to range from 5 to 18.5% in the investigated samples. Aloe-emodin was detected by tlc in the carried-over non-carboxylic genins. Traces of other minor genins were also detected.

From the uv spectrum of aloe-emodin (figure 1), one can predict that its presence in the determinative step would lead to erroneous high results for both sennosides and rhein glycosides.

The perspective of the present procedure was to quantitate the actual sennosides content, abandoning the conventional estimation of total glycosides in terms of sennosides, and to consider important secondary constituents, such as the different rhein glycosides.

The procedure provides a contaminants-free carboxylic genins fraction that is measured at 377 and 430 nm, corresponding to sennidins and rhein maxima (table 1). After proper correction for mutual spectral interference, the results are presented as: 1. total sennosides content, and 2. total rhein glycosides content, calculated as rhein monoglucoside (table 2). Such a presentation of results would help relevant evaluation of the actual activity of the assayed drug.

[5]The reported maximum for sennidin A and sennidin B is at 375 nm and has been found here to be 377 nm.

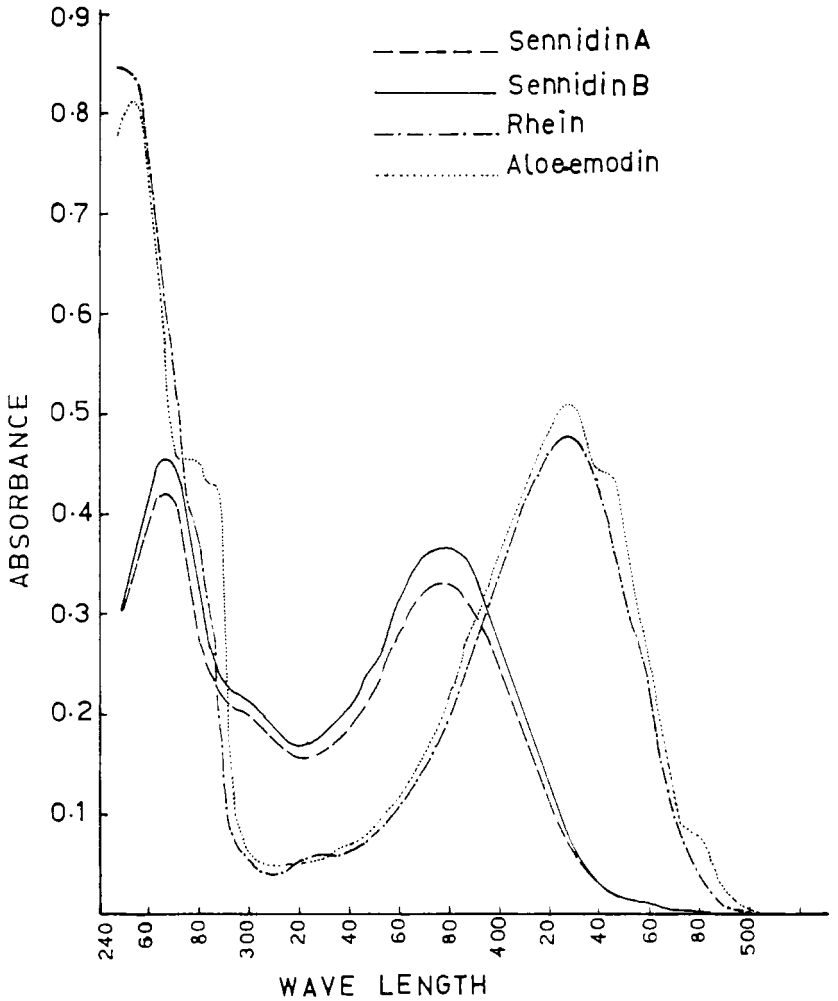

FIG. 1. Uv—absorption spectra of sennidin A, sennidin B, rhein and aloe-emodin in ethyl acetate.

Formulae A and B (below) were derived for the calculation of total sennosides, calculated as sennoside A : B and total rhein glycosides, calculated as rhein monoglucoside, respectively. It is noteworthy here to mention that the 2 : 3 sennoside A : sennoside B ratio determined by Brendel *et al.* (10, 14) was adopted here (table 1).

FORMULA A.—Sennosides content =

$$\frac{A_{377} \times a_{rh;480} - A_{480} \times a_{rh;377}}{a_{sd;377} \times a_{rh;480} - a_{sd;480} \times a_{rh;377}} \times \frac{1.6074 \times 2 \times 10 \times 100}{\text{sample wt. in g} \times 10}$$

$$= \frac{A_{377} \times 47.5 - A_{480} \times 18.5}{35.4 \times 47.5 - 8.2 \times 18.5} \times \frac{1.6074 \times 2000}{\text{sample wt. in g} \times 10}$$

$$= (A_{377} \times 47.5 - A_{480} \times 18.5) \times \frac{210.2}{\text{wt. in mg}}$$

FORMULA B.—Total rhein glycosides (calc. as monoglucoside) =

$$\frac{A_{430} \times a_{sd:377} - A_{377} \times a_{sd:430}}{a_{sd:377} \times a_{rh:430} - a_{sd:430} \times a_{rh:377}} \times \frac{1.57 \times 2 \times 10 \times 100}{\text{sample wt. in g} \times 10}$$

$$= \frac{A_{430} \times 35.4 - A_{377} \times 8.2}{35.4 \times 47.5 - 8.2 \times 18.5} \times \frac{1.57 \times 2000}{\text{wt. in g} \times 10}$$

$$= (A_{430} \times 35.4 - A_{377} \times 8.2) \times \frac{205.3}{\text{wt. in mg}}$$

Where: A_{377} = absorbance of the test solution at 377 nm.
A_{430} = absorbance of the test solution at 430 nm.
$a_{rh:430}$ = absorptivity of rhein at 430 nm.
$a_{rh:377}$ = absorptivity of rhein at 377 nm.
$a_{sd:377}$ = absorptivity of sennoside A:B (2:3) mixture at 377 nm.
$a_{sd:430}$ = absorptivity of sennoside A:B (2:3) mixture at 430 nm.
1.6074 = factor of transformation of sennidins to sennosides.
1.57 = factor of transformation of rhein to rhein monoglucoside.
47.5, 18.5, 35.4, and 8.2 = absorptivity values (table 1).

The proposed procedure, although a bit longer than the procedure of Brendel *et al.* (10), ensures complete initial extraction of the glycosides and complete elimination of non-carboxylic anthracene and flavonoidal genins before the final determinative step. It was also shown to be of reliable reproducibility (table 2) for the estimation of both the sennosides and total rhein glycosides.

TABLE 2. Analysis of some drugs by the proposed method and by Brendels' *et al.* (10) procedure.

Sample	Batch (No.)	Brendels' sennosides %	Proposed method	
			sennosides %	rhein gly. %
Cassia senna:				
a. leaf	(1)	2.730	2.301	0.538
	(2)	2.548	1.866	0.373
b. fruit	(1)	3.959	2.960	0.906
	(2)	2.867	2.138	0.681
Cassia angustifolia:				
a. fruit	(1)	2.184	1.605	0.820
	(2)	2.457	1.960	0.919
	(3)	2.378	1.839	0.785
Calcium sennoside tablets[a]:	(1)	9.555[b]	8.140	0.58
	(2)	10.192	8.507	0.59
Relative standard deviation:[c]			±2.72	±4.7

[a]Pursennid tablets "Sandoz".
[b]Content is given as mg/tablet.
[c]Calculated for Cassia angustifolia fruits (n=6).

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LITERATURE CITED

1. Martindale, "The Extra Pharmacopoeia", 27th Ed., The Pharmaceutical Press, London, 1977, p. 1342, 1343.
2. G. E. Trease and W. C. Evans, "Pharmacognosy", Bailliere Tindall, London, 1978, p. 377, 380.
3. British Pharmacopoeia, Her Majestys' Stationary Office, London, 1973, p. 418.
4. British Pharmaceutical Codex, The Pharmaceutical Press, London, 1973, p. 437, 438.
5. H. Auterhoff and R. Sachdev, *DAZ*, **102**, 921 (1962).
6. K. H. Muller, B. Christ and G. Kuhn, *Arch. Pharm.*, **295**, 41 (1962).
7. W. Kussmaul and B. Becker, *Helv. Chim. Acta*, **30**, 59 (1947).
8. J. Lemli, *Verhand. Konikt. Vharm. Acad. Genresk. Belg.*, **25**, 458 (1963).
9. H. Frederich and S. Baier, *Planta Med.*, **23**, 74 (1973).
10. W. D. Brendel and D. Schneider, *Planta Med.*, **25**(1), 63 (1974).
11. J. W. Fairbairn, C. A. Friedmann and A. H. Ryan, *J. Pharm. Pharmacol.*, **10**. Suppl. 186T–192T (1958).
12. J. W. Fairbairn and A. B. Schrestha, *Phytochemistry*, **6**, 1203 (1967).
13. H. Auterhoff and Kinsky, *Arch. Pharm.*, **298**, 810 (1965).
14. W. D. Brendel and D. Schneider, *Planta. Med.*, **25**(4), 342 (1974).
15. W. D. Brendel and L. Berger, *Planta. Med.*, **26**(2), 124 (1974).
16. R. H. Thomson, "Naturally Occurring Quinones", Academic Press, London and New York, 1971, p. 399, 402.
17. Y. Yamaguchi, "Spectral Data of Natural Products", vol. I, Elsevier Publishing Company, Amsterdam, London, New York, 1970, p. 432.