VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART X. A NEW ACTIVE GLYCOSIDE OF SENNA

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Evidence is produced to show that the sennosides are probably breakdown products from primary glycosidal compounds occurring in the crude drug. Carefully prepared extracts have been shown to contain these primary compounds and one such compound has been isolated in a pure form. Biological and chemical assays show it is about 50 per cent more active than the sennosides and the relation of this fact to the explanation of the total activity of senna is discussed.

IN an earlier paper in this series Fairbairn and Saleh[1] have shown that between 60 to 70 per cent of the biological activity of senna pod and senna leaf (*Cassia acutifolia* Delile and *C. angustifolia* Vahl.) can be accounted for by the content of sennosides A and B and a third non-rhein anthracene glycoside present, when these are estimated as sennosides by the method of Fairbairn and Michaels[2]. The remaining 30 to 40 per cent of the activity is due to some unknown factor which could be extracted from the crude drug along with the anthracene glycosides, by hot water and by 70 per cent ethanol, but none of it survived if an aqueous solution was concentrated by prolonged evaporation as directed in the B.P. 1953 for Liquid Extract of Senna[3]. The 30 to 40 per cent activity unaccounted for might be explained by any of the following possibilities. (*a*) The amount of the third glycoside present is larger than reported earlier due to faulty methods of extraction or destructive methods of hydrolysis during assay. (*b*) There is present in the crude drug some non-anthracene compound which exerts a laxative effect. A possibility would be the flavonol glycoside, kaempferin, reported to be present in senna leaf and pod and said to exert a laxative effect[4]. (*c*) The sennosides themselves are breakdown products from primary compounds which may be more active. An analogous situation occurs in such drugs as aconite and digitalis, where fragments of the primary active principles are sometimes broken off during extraction with consequent loss of activity.

The following experiments were therefore designed to investigate these possibilities.

EXPERIMENTAL METHODS AND RESULTS

Preliminary work on the separation and nature of the third (non-rhein) glycoside and its chemical and biological assay, showed that the extra biological activity could not be accounted for by this glycoside since the amounts present in the crude drug were not greater than those previously reported[1]. The first suggested explanation may therefore be ruled out. Of the remaining two explanations that based on the presence of sennoside precursors seemed more likely. Chemical and biological assays of a

large number of samples of senna pod show a close correlation between sennoside content and biological activity[5], which suggests that the latter is associated with the sennosides. To confirm this it was necessary (*a*) to use the mildest condition of treatment possible to minimise breakdown of the postulated primary substance, and (*b*) to separate the acidic glycosides (sennoside-type, based on rhein) from the non-acidic glycosides, such as the non-rhein (third) glycoside and kaempferin. The following further experiments were therefore carried out.

The Preparation of Crude Fractions

Ethanol (70 per cent) extracts of powdered senna pod were prepared in the cold with and without the presence of dilute mineral acid. The rhein glycosides were separated from the non-acidic either by precipitation of the former as calcium salts or by evaporating the ethanol extract to a dry powder in vacuum and removing the non-acidic glycosides from the powder with anhydrous methanol. The rhein fractions from both extracts were then assayed chemically and biologically and the results are shown in Table I.

TABLE I

Analyses of one crude fraction made with neutral ethanol and another with acidic ethanol

Solvent	Chemical assay expressed as mg. sennosides A and B/g.	Biological assay activity equivalent to sennosides A and B (mg./g.)	B/C ratio*
Neutral ethanol (70 per cent)	260	397 (248–466)**	1·5
Ethanol (70 per cent) containing 0·5 per cent HCl	327	385 (313–473)**	1·2

* Biological assay result, expressed in terms of sennosides, divided by chemical assay result expressed in the same terms. With pure sennosides the ratio would be unity.
** Limits of error, P = 0·95.

It will be seen that when acidic ethanol was used for extraction the biological activity was not much higher than would be expected if sennosides only were present in the resulting extract. On the other hand, when neutral ethanol was used the resulting extract was 50 per cent more active than would be expected if sennosides only were present. These experiments therefore indicate that the neutral extracts may contain the sennoside precursors and that the use of mineral acid during extraction and concentration should be avoided. They further confirm the suggestion that the enhanced activity of the crude drug is associated with the sennosides rather than with the non-rhein and flavonol glycosides, which were separated from the fractions tested in the above experiments. The following two procedures indicated beyond doubt that enhanced activity was associated with the sennosides.

Isolation of a New Glycoside

(*a*) By Acetylation

Powdered senna pod (100 g.) was stirred with cold neutral ethanol (70 per cent; 1200 ml.) for 8 hours. After filtration, the cake was washed

with a further quantity (400 ml.) of the same solvent and the ethanolic extract was concentrated to small volume in a rotary vacuum still at below 30° and then to dryness in a vacuum desiccator, the final stages being over P_2O_5. The resulting dry powder (25 g.) was stirred with anhydrous methanol (2 × 200 ml.) which removed chlorophyll, practically all the non-rhein anthraquinone (third) glycoside and the kaempferin. The residue (18 g.) was collected, washed with a little ether and dried in a desiccator after which it was dissolved in pyridine (180 ml.) and acetic anhydride (90 ml.) and heated to 100° for 3 hours. Most of the solvent was removed under reduced pressure and the residue taken up in ethyl acetate, which was washed with a little 0·1 N hydrochloric acid and then with water until it was neutral. The ethyl acetate layer was shaken with several portions of 20 per cent potassium hydrogen carbonate solution in order to extract the acidic (acetylated) glycosides; the alkaline solution was weakly acidified with dilute hydrochloric acid and immediately re-extracted into ethyl acetate. This was then washed with water, dried over anhydrous sodium sulphate and concentrated to small bulk (35 ml.).

The ethyl acetate solution was transferred to a silica gel column and eluted with a benzene-ethyl acetate mixture (3:1) until the eluate was colourless. This fraction contained the bulk of the acetylated primary glycosides originally present in the dry extract of the pod. Further elution of the column with mixtures containing various proportions of benzene and ethyl acetate yielded small amounts of anthraquinone compounds which remain to be investigated. The main fraction was evaporated to dryness *in vacuo* and dissolved in about 30 ml. of warm ethyl acetate. Light petroleum (40 to 60°) was cautiously added till the solution became turbid. It was then warmed till clear and allowed to cool slowly until crystallisation set in. The crystals were dissolved in the same solvent and treated by the above chromatographic procedure and re-crystallised, until no further changes occurred in the ultra-violet spectrum.

This acetyl substance was shown to be different from acetyl sennoside A and acetyl sennoside B by ultra-violet and infra-red spectra, anthraquinone assay and equivalent weight determinations. The latter gave a value of 960 which indicates that the new substance is a larger molecule than sennoside. It is hoped to publish further details later. Biological assays showed that neither this new acetyl compound nor the acetyl sennosides had any purgative activity. The new acetyl compound was therefore de-acetylated by means of potassium hydroxide in methanol. The resulting product was washed with butanol to remove any unchanged acetyl glycoside but further purification, by removal of excess potassium acetate, was not attempted as this substance would not be likely to interfere with the chemical or biological assays. The usual chemical assay process[2] was carried out on this product which behaved in the process exactly like the sennosides and gave a figure of 46 mg./g., as sennosides. Biological assay, however, showed it had an activity equivalent to 92·8 mg. of sennosides per g. This work therefore showed that senna pod contains a rhein-type glycoside whose acetyl derivative

differs markedly from those of the sennosides and whose biological activity is considerably greater than that of the sennosides. An attempt was therefore made to isolate this material by a more direct method.

(*b*) *By Direct Extraction*

Powdered senna pod (100 g.) was extracted with ethanol (70 per cent) as described in (*a*). The combined ethanolic extracts were stirred with 4 g. anhydrous calcium acetate for 15 minutes. Precipitation of calcium salts was completed by the addition of 1700 ml. of acetone and the combined calcium salts were collected and dried in a vacuum desiccator. The product (10 to 15 g.) was dissolved in water (150 to 200 ml.) and stirred with Zeo-Karb 225 (H^+ cycle; about 20 to 30 g.) until the solution was free from metal ions. The aqueous solution, which contained practically all the primary glycosides initially present, was filtered from resin and evaporated below 30° under reduced pressure to about 20 ml. *iso*Propanol (100 ml.) was added and the resulting yellow precipitate collected and dried over P_2O_5 at room temperature. It was then dissolved as completely as possible in 85 per cent *iso*propanol (150 ml.) and the solution transferred to a cellulose column, which was eluted further with 85 per cent *iso*propanol until the eluate was colourless. The latter was concentrated under reduced pressure to a syrup and *iso*propanol (4·5 volumes) added. The resulting yellow precipitate was recrystallised several times from 90 per cent *iso*propanol. The following is a summary of its properties and differences from the sennosides.

Properties of the New Glycoside and Differences From the Sennosides

Physical Properties

The new glycoside is dull yellow and very soluble in water; in contrast, both sennosides A and B are bright yellow and only slightly soluble in water. It does not crystallise from aqueous acetone or aqueous 2-ethoxyethanol as used by Stoll and co-workers for the sennosides[6].

Physical constants. *M. p.* 157 to 159° (sennoside A decomposes at 200 to 240°, sennoside B melts at 180 to 186°)[6].

Equivalent weight. By potientiometric titration in 80 per cent ethanol, about 620 (sennosides 432).

Molecular weight. Determinations by the isopiestic method using sennoside A as reference substance and 70 per cent ethanol as solvent gave a molecular weight of 1164 (sennosides 862).

Chromatography

Ascending paper chromatography using 85 per cent *iso*propanol as running solvent gave an R_F value for the new glycoside of 0·9 to 1·0, whereas the sennosides remained at the origin.

Hydrolysis and Chemical Assay

When a methanolic solution containing a trace of mineral acid is warmed at about 60° for 10 to 15 minutes and allowed to cool overnight

yellow crystals are formed. These can be recrystallised from 50 per cent acetone and shown to be sennoside by comparison with authentic material.

When hydrolysed and assayed by the normal method[2] the final red solution had an absorption curve the same as that produced by the sennosides when treated similarly. The extinction coefficient of the new glycoside, however, was only 74 per cent of that of sennoside; this corresponds to a sennidine content of 49·3 per cent (sennoside affords 62·5 per cent sennidine).

Chemical and Biological Assays

The results recorded in Table II show that the new glycoside is about 50 per cent more active than the sennosides.

TABLE II

CHEMICAL AND BIOLOGICAL ASSAYS OF THE NEW GLYCOSIDE

Chemical assay expressed as sennosides A and B (mg./g.)	Biological assay activity equivalent to sennosides A and B (mg./g.)	B/C ratio*
743	1130 (1035–1233) Limits of error, P = 0·95.	1·52

* See note to Table I.

DISCUSSION

The total activity of senna pod. These experiments suggest that the sennosides are break-down products of primary glycosides. It has not yet been established whether sennoside A and sennoside B are derived from two separate primary glycosides or from one, but sufficient evidence has been produced to show that at least one primary glycoside is present and that it is about 50 per cent more active biologically than the sennosides (when compared on the basis of sennoside content). If it can be shown that the bulk of the rhein glycosides of senna pod are of this primary type then the remaining 30 to 40 per cent activity referred to in the Introduction will be largely accounted for. Hence the total activity of senna pod could be accounted for by the presence of these primary glycosides together with small amounts of secondary substances and of non-rhein anthracene glycosides. Methods for the chemical estimation of these various compounds are being investigated in order to establish these suggestions on a quantitative basis.

Senna extracts on storage. It has been reported that certain senna extracts, on storage, lose biological activity more rapidly than is revealed by the chemical assay process. This could be explained by assuming that the primary compounds are slowly changed into less active secondary glycosides, which would nevertheless give the same chemical assay figure. Experimental evidence for this assumption has been collected and will be presented at a later date. It has been observed that dry powdered senna pod on the other hand, retains its biological activity over many years.

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REFERENCES

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4. Fukuda, *Arch. exp. Path. Pharmak.*, 1932, **164**, 685.
5. Fairbairn, in *The Pharmacology of Plant Phenolics*, Academic Press, London, 1958.
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DISCUSSION

The paper was presented by DR. C. A. FRIEDMANN.

THE CHAIRMAN. In view of the isolation of the new primary glycoside was the synergistic hypothesis still accepted? He would have liked to have seen figures for the elementary analyses of the new glycoside. Did the Authors conclude that the chemical assay of senna was useless for judging the biological potency?

DR. W. MITCHELL (London). If the neutral alcohol extract contained the postulated more active primary glycoside why was its biological activity not greater than the extract made with acidified alcohol? Why did the two crude products give essentially the same chemical assay? Did the acidified ethanol extract contain additional biologically inert anthraquinone derivatives? Was the new glycoside crystalline?

MR. C. A. JOHNSON (Nottingham). Were the Authors satisfied that the glycosides obtained by the gentle and the drastic extractions were identical? What were the limits of error in the biological assays? If 600 mice were used, it would probably be ± 15 per cent when the B/C ratio of 1·2 might not be significantly different from 1 and if less animals were used it might not be significantly different from 1·5.

DR. J. B. STENLAKE (Glasgow). The acetylation procedure would not harm the glycosides. He suggested the use of dry ammonia-methanol which would rapidly hydrolyse acetyl groups to give chloroform-soluble acetamide.

DR. J. M. ROWSON (Ibadan). Was Alexandrian or Tinnevelly pod used and had the Authors compared the two varieties? Had chromatographic methods been tried for the estimation of the primary glycoside? Was this considered to resemble either sennoside A or B with two glucose molecules attached, which would give a difference in molecular weight?

DR. FRIEDMANN replied. Synergism still prevailed with the crude primary glycoside. Details of the carbon and hydrogen figures were available and the exact character of the sugar was being investigated. They were also working on another chemical assay which they hoped would enable them to dispense with the biological assay. Their present chemical assay could not be used to determine the biological activity of senna. The material was obtained in a crystalline form from 90 per cent

*iso*propanol. The acetylation technique had been shown by Muhlemann not to affect complicated glycosides. The main work was carried out on Alexandrian pod, but had been repeated on other varieties and on leaf. Only preliminary experiments had been carried out with chromatographic methods. It was fairly certain that there were extra glucose molecules attached and these would account for the increased molecular weight.

DR. FAIRBAIRN replied. The explanation of why the two extracts varied chemically was that the use of an acid solvent gave a product with less inert material; the glycosides were therefore present in higher concentration. The chemical assay was obviously the correct base-line to use in examining these extracts; where the biological potency was higher than would be expected from the chemical assay, it is reasonable to assume that there is a difference in the glycosides present. His experience of the biological assay process would lead him to expect the mean of four assays to vary by not more than $\pm$ 10 per cent from the true mean. The first column of Table II records the chemical assay, expressed as sennosides A and B, as 743 mg./g. If the glycosides were pure sennosides and if four biological assays were made, the mean should fall between 670 and 820 mg./g. The figure shown in the last column was based on four biological assays and it will be seen that the mean and limits of error 1035 to 1233 show a significantly higher value for potency.