agents retard healing by their anti-inflammatory and inhibitory actions on mucopolysaccharide synthesis (2). Retinoic acid reverses the healing retardation action of anti-inflammatory agents (13). Active retinoic acid homologs studied in the present paper promote wound healing by increasing granuloma mass.

The healing-promoting action of these active retinoic acid homologs is further evidenced by the increase of hydroxyproline and hexosamine contents of the granuloma (Tables III–VI). Hydroxyproline is an important constituent of collagen, which is an essential component of connective tissue. Hexosamine is a component of mucopolysaccharide, and the role of mucopolysaccharide in healing was extensively discussed previously (2). As shown in Tables III–VI, both the hydroxyproline and hexosamine contents were increased in the granuloma with either one of the active compounds. This fact indicated that these compounds increased connective tissue regeneration. However, the amount of hydroxyproline or hexosamine per unit of weight of granuloma of the experimental was lower than that of the control. This fact indicated that these active compounds also stimulated inflammation.

It is suggested that active retinoic acid homologs, like retinol, promote healing by inducing inflammation and increase mucopolysaccharide and collagen synthesis mechanisms of action.

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Extraction and Separation of Anthraquinone Glycosides

STEPHEN CHAO YUNG SU and N. M. FERGUSON▲

Abstract □ A method was developed for extracting anthraquinone aglycones and their corresponding glycosides from plant materials and for separating them in pure form. The method consists of rendering the glycosides and aglycones insoluble in chloroform and then removing, stepwise, the interfering extractable substances. The method makes use of the fact that the aglycones are chloroform soluble whereas the glycosides are not. The method was applied with good results to the isolation of the aglycones and glycosides of cascara and senna.

Keyphrases □ Anthraquinone aglycones and corresponding glycosides—extraction, separation from plant material □ Cascara bark—extraction, separation of anthraquinone aglycones and glycosides □ Senna leaves—extraction, separation of anthraquinone aglycones and glycosides

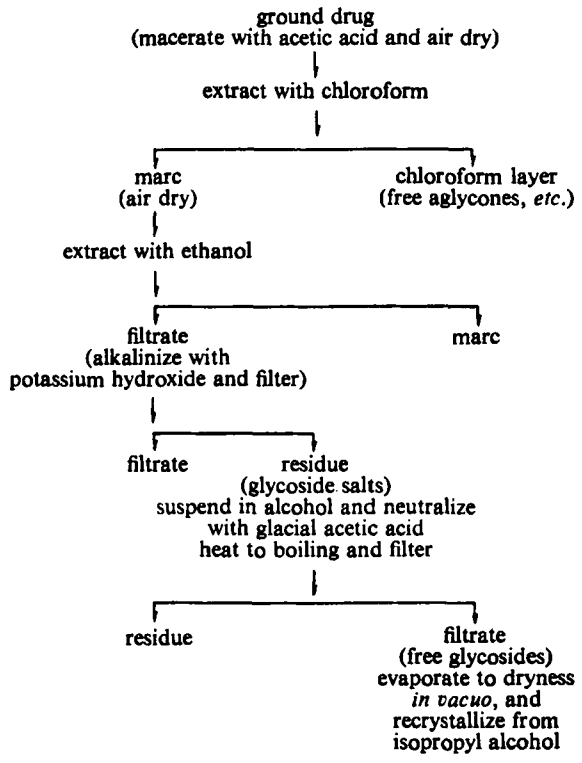
The methods that have been used to date for the isolation and purification of the anthraquinone derivative glycosides from cascara bark and senna leaflets fall into three categories. The first involves an aqueous or hydroalcoholic menstruum (1), and the second uses a pure solvent such as methanol (2, 3), ethanol (4), propyl alcohol, or isopropyl alcohol (1). The final method uses a solvent such as chloroform or ether (5) to remove certain interfering substances, and then extraction proceeds using an alcoholic menstruum. Purification of the various fractions is then accomplished by recrystallization from one of these solvents. In some procedures, the fractions are acetylated in benzene (1, 3, 6) prior to being further purified by fractional crystallization.

In view of the fact that interfering plant constituents in cascara bark and senna leaflets made a clearcut separation of their constituents impossible by these methods, it was decided to try to develop a new procedure which would produce purer fractions of the anthracene derivatives, particularly derivatives of hydroxyanthraquinones, both free and in the form of glycosides.

EXPERIMENTAL

Extraction Procedure—The extraction procedure chosen (Scheme I) is based on the fact that the hydroxyanthraquinone derivatives and their corresponding glycosides are present both free and as magnesium, potassium, and sodium salts combined either through a hydroxy or a carboxylic acid group. In addition, the separation method capitalizes on the differential solubility in chloroform of the glycosides and their aglycones as well as other anthracene derivatives. The glycosides are insoluble in chloroform whereas the free aglycones, as well as the other anthracene derivatives, are chloroform soluble.

The extraction procedure consisted of macerating the ground drug for 24 hr. with 5% acetic acid. The purpose of this step was to liberate the free anthraquinones and their corresponding glycosides from their magnesium, potassium, or sodium salts, in which form



Scheme I—General Extraction Procedure

some of them are present in the plant. The maceration mixture was then dried and extracted with chloroform until free of anthracene derivatives. The drug was again air dried and then extracted with warm 95% ethanol. The alcoholic extract was then treated with 5% alcoholic potassium hydroxide solution until precipitation of the anthraquinone glycosides was complete. Following this step, the residue was filtered and washed with 95% ethanol to remove the last traces of potassium hydroxide and was then sucked dry on a Büchner funnel. The anthraquinone glycoside salt residue was suspended in warm ethanol, and the free glycosides were liberated by adding glacial acetic acid dropwise with stirring until the color

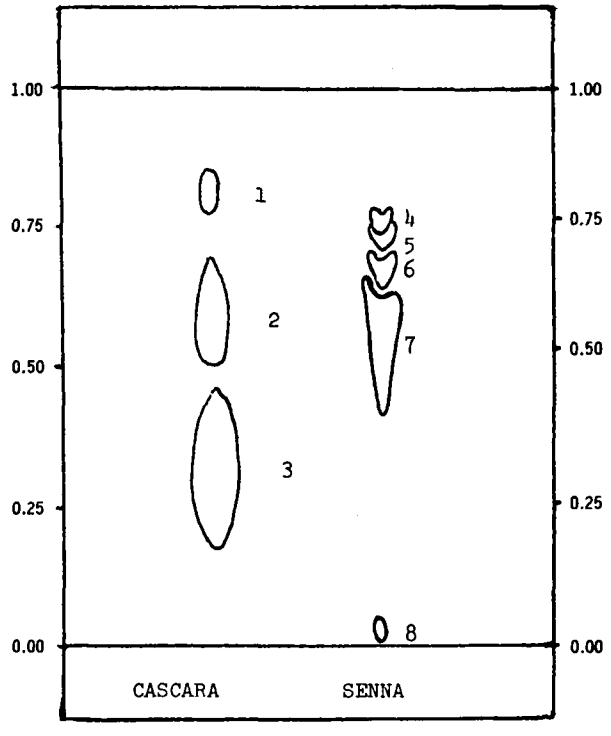


Figure 1—*Chromatogram of glucosides.*

Table I—R_f Values of Cascara Glucosides Calculated from Fig. 1

Spot Number	R_f Value
1	0.80
2	0.57
3	0.26

Table II—R_f Values of Senna Glucosides Calculated from Fig. 1

Spot Number	R_f Value
4	0.75
5	0.71
6	0.65
7	0.48
8	0.05

change was complete. The solution was then filtered hot, and the residue was further extracted with warm ethanol. The combined filtrates were then evaporated to dryness under reduced pressure, and the residue was recrystallized from isopropyl alcohol.

Cascara Sagrada—Powdered cascara sagrada bark (*Rhamnus purshiana*, D. C.)[1], 300 g., was macerated with 850 ml. of 5% acetic acid for 24 hr. in a 2000-ml. stoppered conical flask. The mixture was air dried, after which it was exhaustively extracted with chloroform to remove free anthraquinones as well as many impurities. This required 11 600-ml. portions of chloroform. The absence of a red color imparted to a small portion of the chloroform filtrate on the addition of a few drops of 5% alcoholic potassium hydroxide indicated complete extraction of the hydroxyanthraquinones. The chloroform extracts were then combined and evaporated in air to give 21.6 g. of residue.

The marc obtained was air dried and extracted exhaustively with 600-ml. portions of warm ethanol until successive extracts showed no red or pink coloration when treated with a few drops of a 5% alcoholic potassium hydroxide solution. For complete extraction, 8100 ml. of ethanol was required. The combined filtrates were then evaporated to 3000 ml. *in vacuo* at 40°. To this solution, 5% alcoholic potassium hydroxide was added until no further precipitation occurred. The solution was then filtered, and the residue was washed with cold ethanol to remove the last traces of potassium hydroxide and then dried by suction on the Büchner funnel. The residue thus obtained, consisting of the potassium salts of the glycosides, was suspended in warm ethanol; the free glycosides were liberated by adding glacial acetic acid slowly with stirring. The solution was then filtered hot, and the residue was further extracted with warm ethanol. The combined filtrates were evaporated to dryness *in vacuo* at 40°, and the residue thus obtained was recrystallized from isopropyl alcohol. The yield of glycosides from 300 g. of cascara bark was 5.5 g. or 1.83%.

Senna—Powdered senna[2] (*Cassia angustifolia*, Delile), 300 g., was extracted by the same method as was used for cascara bark. The chloroform extracts, when combined and evaporated in air, gave 22.4 g. of residue. The yield of glycosides from the marc was 5.2 g. or 1.73%.

Chromatographic Separation—*Glucosides of Cascara and Senna*—Because of the difference in the solubility of the hydrophilic anthraquinone glycosides compared to that of the lipophilic aglycones, it is impossible to carry out the separation of both these compounds simultaneously in one system. It became necessary, therefore, to use two different sets of conditions for their separation.

In the chromatographic separation of the glucosides, the method of Betts *et al.* (7) was used. This consisted of using a mixture of water, acetone, and benzene (2:1:4); the mixture was shaken vigorously and then allowed to separate. The lower aqueous phase (*i.e.*,

[1] Cascara sagrada bark USP XVI, 100 mesh. Control No. BKP 1853, S. B. Penick and Co., New York, N. Y.

[2] Senna leaves, *Cassia angustifolia*, Delile, Tinnevelley No. 1, NF X, 40 mesh. Lot No. WEB-414, E-10317, S. B. Penick and Co., New York, N. Y.

Table III—R_f Values of Aglycones Calculated from Fig. 2

Aglycone	Spot	Pure Aglycone R_f Value	Cascara Observed R_f Value	Senna Observed R_f Value
Chrysophanic acid	A	0.98	0.98	0.98
Aloe emodin	B	0.83	0.83	0.83
Frangula emodin	C	0.53	—	0.53
Rhein	D	—	0.035	—

the running solvent) and a beaker containing the upper phase of the mixture were placed on the bottom of a chromatographic jar. After equilibrium was established, the glucoside mixture, dissolved in alcohol, was spotted on Whatman No. 1 paper and placed in the jar. When the solvent front reached a suitable height, the paper was removed, dried, sprayed with 0.5% magnesium acetate in methanol, and heated to about 100° for 3–5 min. The glucosides appeared as yellow to orange spots. The chromatograms are shown in Fig. 1, and the R_f values are listed in Tables I and II.

In the chromatogram for the mixture of the glucosides of cascara, three distinct spots developed on the paper, showing three separate cascara glucosides. The spot marked "1" and having an R_f value of 0.80 corresponds to Frangula emodin glucoside, the spot marked "2" and having an R_f value of 0.57 corresponds to Aloe emodin glucoside, and the spot marked "3" and having an R_f value of 0.26 corresponds to chrysophanic glucoside.

In the chromatogram for the mixture of the glucosides of senna, it is seen that five spots appear for senna corresponding to five separate glucosides.

Aglycones of Cascara and Senna Glycosides—To determine the exact composition of the glycosides of cascara and senna as extracted by the developed procedure, it was thought advisable to hydrolyze each of the glycosidal fractions thus obtained and to identify the aglycones.

Hydrolysis of Glycosides of Cascara and Senna—A solution consisting of 20 mg. of the appropriate isolated glycosidal fraction was dissolved in 25 ml. of a 1:1 methanol–water mixture and refluxed in a boiling water bath for 30 min., using a mixture of hydrochloric acid and ferric chloride as the hydrolytic agent (7, 8). The mixture was then cooled and extracted with ether, using three 25-ml. portions. The combined ether fractions were then washed with three 15-ml. portions of water to remove the hydrochloric acid and ferric chloride. The ether solution was diluted to 50 ml. with ether and dried over anhydrous sodium sulfate.

Chromatographic Separation of Aglycones—A small amount of the filtered ether solution of the aglycones was spotted on Whatman No. 1 paper and chromatographed by the ascending method according to Betts *et al.* (7), using toluene as the running solvent.

The aglycone chromatograms are shown in Fig. 2. The aglycones hydrolyzed from the glycosides extracted and the R_f values for these aglycones are listed in Table III, where they are compared with published values for the pure aglycones. It is seen that the R_f values of the pure aglycones are just reversed in value when converted to their corresponding glucosides. This is due to the fact that, in the polar solvent system used, the addition of the sugar to the aglycone as a glucoside increases the polarity of the molecule. Thus, chrysophanic acid has an R_f value of 0.98, whereas chrysophanic acid glucoside has an R_f value of 0.26. Similarly, Aloe emodin has an R_f value of 0.83 and Aloe emodin glucoside has an R_f value of 0.57. Frangula emodin shows an R_f value of 0.53, whereas the corresponding Frangula emodin glucoside R_f value is 0.80. This general principle is borne out in the literature (8). The literature (8) value given as the R_f for rhein is 0.00–0.03, since there is considerable tailing in this constituent. The results obtained here of 0.035 are certainly within experimental error for this determination.

The paper chromatograms, after being developed through spraying, were dipped in melted paraffin to preserve them. The chromatograms then could be stored for long periods without loss of color and could serve as references for comparison with later work.

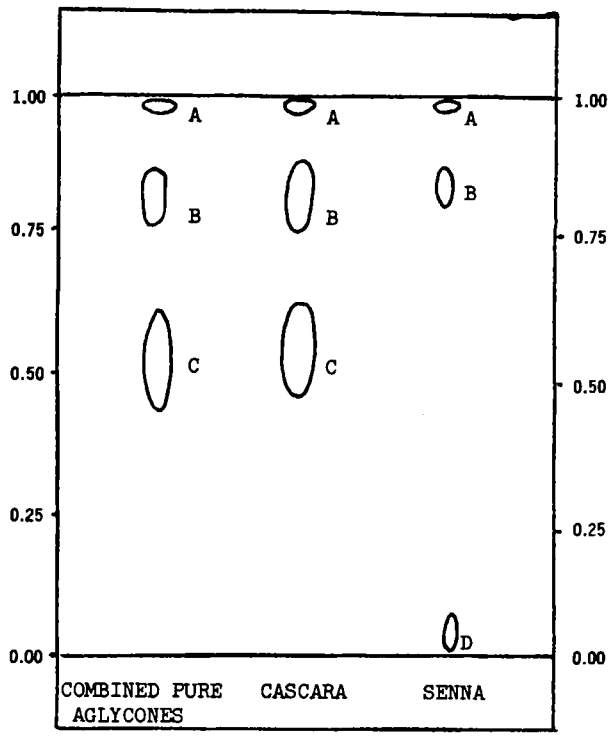


Figure 2—*Chromatogram of aglycones.*

SUMMARY AND CONCLUSIONS

A method was developed for extracting anthraquinone aglycones and their corresponding glycosides from plant materials and for separating them in pure form. This method eliminates the difficulties of filtration and extraction encountered in previous methods by rendering the glycosides insoluble from the start. In this way, many interfering substances are eliminated. Paper chromatograms and corresponding R_f values were tabulated for the aglycones and glycosides of cascara and senna.

A method was also developed for the preparation and preservation of paper chromatograms.

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