(Z.M.F.4.C.) under the following operating conditions. Calibration is made with a quinine sulfate solution (5); 1 mm. monochromator's slits are used. The sensitivity of the instrument is set so that a full scale deflection of the galvanometer spot is obtained for 3.5 mcg./ml. concentration of panthenol in reading the diluted solution, using quartz cells—1 cm. light path—for the measurements.

F.—Calculate the amount of panthenol in the unknown syrup by

$$\frac{Ps\,(Iu - Ib)}{Vu\,(Is - Ib)} \times 200 = \text{mg. panthenol in 100 ml. syrup}^1$$

where Iu = fluorescent intensity of the unknown, Is = fluorescent intensity of the standard. Ib = fluorescent intensity of the blank, Ps = standard panthenol content in mg./100 ml., and Vu = volume of the sample of the unknown syrup.

RESULTS AND DISCUSSION

In this laboratory the described method has given reproducible results, both with multivitamin syrups and tablets. With the latter, the first step of the procedure was neglected: the tablets were directly powdered and extracted as described in step *B*.

As shown in Table I, the mean deviation between the observed results was ±2% which indicates a good reproducibility in the range of concentration studied.

As mentioned by Zappala and Simpson (3), the recoveries of panthenol after treatment on ion-exchange resins are low because of a partial retention of the vitamin by the resins. Several assays with internal standards have shown that the mean retention in our experimental conditions was approximately 6% of the total panthenol content of the solution. A correction factor of 6% was thus introduced in the final calculation of panthenol in the syrups and tablets.

The quantitative determination of β-alanol in the final hydrolyzate could have been made by one of the methods described in the literature (1, 3, 4). It seemed interesting to use the new fluorometric method perfected by Close, *et al.* (7), which is routinely used in our laboratory for other purposes. This method is easy, rapid, precise, and very sensitive.

As illustrated in Fig. 1, the relationship between the fluorescent intensity and the β-alanol concentration is perfectly linear in a range of concentration between 0.1 and 1 mcg./ml. of β-alanol in the diluted solution (or 0.27 and 2.7 mcg./ml. of panthenol). This range is highly convenient for the needs of the quantitative estimation of panthenol in the pharmaceutical preparations.

We believe that the method proposed can be used as a routine procedure for the panthenol determination in the pharmaceutical preparations with a high sugar content, and that it can also be the basis for the assay of the other vitamins contained in the same preparations.

[1] Uncorrected value; see next paragraph for the correction factor.

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Two New Glucosides from *Cassia angustifolia* Pods

By M. L. KHORANA and M. M. SANGHAVI

Fractional precipitation and chromatographic studies have shown that *C. angustifolia* pods contain, besides sennosides A and B, glucosides of rhein and chrysophanic acid. The latter were best isolated by acidification of the aqueous extract to pH 3. Biologically, a mixture of these new anthraquinone glucosides and the bianthranol glycosides was more active than either. The possibility of the presence of traces of aloe-emodin or emodin glucoside has also been indicated.

In 1949, STOLL (1) isolated sennosides A and B from the leaves and pods of senna (*C. angustifolia* Vahl and *C. acutifolia* Delile). However, Fairbairn (2) showed that these two glycosides did not represent the full potency of the drug. Further studies of the drug with the purpose of isolating other principles, representing the residual potency of the drugs, were therefore warranted. During recent years *C. acutifolia* has been studied by Fairbairn (3) and Vickers (4), and isolation of a number of glycosides has been claimed.

This work deals with similar studies on *C. angustifolia* pods, and evidence for the presence of two other glycosides is presented.

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EXPERIMENTAL

Aqueous Extract

A coarse powder prepared from 600 Gm. of senna pods was divided into three portions. One portion, after maceration with 36 ml. of 1 *N* sodium bicarbonate and menstruum (water saturated with chloroform), was percolated with the menstruum to obtain 2 L. of the percolate. The second and third portions of the drug were similarly extracted, using first the extract from the previous batch as

TABLE I.—CHROMATOGRAPHY OF *C. angustifolia* GLUCOSIDES

Substance	Spot No.	R_f	Color	
			Visible	Ultraviolet Light
Sennoside A	1	0.00	Yellow changing to brown	Bright yellow
Sennoside B	2	0.30	Yellow changing to brown	Bright yellow
Acid precipitate	3	0.35	Yellow changing to brown	Bright yellow
	4	0.44	Yellow changing to brown	Bright yellow
	5	0.99 Tailing to ...	Reddish brown	Reddish brown

menstruum supplemented by fresh menstruum as required to obtain 2 L. of the percolate from each portion. Two liters of percolate were thus obtained from 600 Gm. of the drug.

Fractionation of the Aqueous Extract

(a) **Acid Precipitate.**—The aqueous extract acidified to pH 3 with concentrated hydrochloric acid and preserved with chloroform was allowed to stand overnight. The acid precipitate (*a*), separated by vacuum filtration through filter paper supported on muslin, was washed with water and dried; yield, 3.7%.

(b) **Neutral Precipitate.**—Sufficient ammonium chloride and anhydrous calcium chloride were added to the combined filtrate and washings from (*a*) to obtain concentrations of 2 and 5%, respectively; pH was adjusted to 7 with liquor ammonia. The yield of the neutral precipitate after filtration, washing with water, and drying, was 2.1%.

(c) **Alcoholic Precipitate.**—Sufficient 95% alcohol was added to the combined filtrate and washings from (*b*) to cause complete precipitation. The yield of the precipitate after filtration, washing with alcohol, and drying, was 13.8%.

Chromatographic Studies of the Acid Precipitate

The acid precipitate (*a*) (0.2 Gm.) was extracted with 15 ml. of 70% methanol; the filtered extract was spotted on Whatman filter, chromatographic grade 1. Solutions of sennoside A in cellosolve and sennoside B in 70% methanol were spotted for comparison. The chromatogram was developed by the ascending technique with 85 ml. of absolute isopropanol and sufficient 1.94 *N* acetic acid to make 100 ml. The chromatograms, sprayed with 1 *N* alcoholic sodium hydroxide, were examined in visible as well as ultraviolet light. The results are given in Table I.

A comparison of colors in visible and ultraviolet light as given in Table I indicates that Spots 3 and 4 of the acid precipitate are most probably due to glycosides. Comparison of R_f values indicates that these spots are due to compounds other than sennosides A and B. Spot 5, because of its color, seemed to be due to free anthraquinones or some coloring matter.

Identification of the New Aglycones

Hydrolysis of the Acid Precipitate.—The acid precipitate (8 Gm.), extracted with ether to remove free anthraquinones, was hydrolyzed by heating with 40 ml. of 3 *N* hydrochloric acid in a boiling water bath for 15 minutes. The anthraquinones from the cooled hydrolyzate were extracted with ether and the aqueous layer set aside for *Identification of Sugars*. The ether extract, after being washed twice with distilled water and dried over sodium sulfate, was concentrated to about 5 ml.

TABLE II.—CHROMATOGRAPHY OF TWO NEW AGLYCONES FROM *C. angustifolia* PODS

No.	Substance Name	R_f	Color
A	Aloe-emodin	0.94	Pink
B	Emodin	0.88	Pink
C	Chrysophanol	0.98	Pink
D	Rhein	0.00	Pink
E	Sennidin A	0.00	Yellow[a]
F	Sennidin B	0.00	Yellow[a]
G	Benzene concentrate	0.98	Pink
H	Alcoholic concentrate	(*1*) 0.00 (*2*) 0.52 (*3*) 0.98	Pink
I	C + G	0.98 (*1*) 0.00	Pink
J	D + H	(*2*) 0.52 (*3*) 0.98	Pink

[a] Yellow slowly changing to violet.

Fractionation and Chromatography of the Ether Extract.—The concentrated ether extract was extracted serially with (*A*) 1 *N* sodium bicarbonate, (*B*) 5% sodium carbonate, and (*C*) 1 *N* sodium hydroxide. The sodium carbonate extract (*B*), had a color that was too weak and was not studied further. Each of the other two alkali extracts was made acidic with hydrochloric acid and re-extracted with ether. After washing the ether extracts with water, the solvent was removed; the residues were repeatedly extracted with hot, sodium-dried, benzene, using 5 ml. each time. The extracts were concentrated to 5 ml. and poured on columns of $CaSO_4 \cdot {}^1/_2\, H_2O$ (E. Merck). The columns were prepared and washed with benzene.

The sodium bicarbonate–soluble fraction (*A*) gave a yellow band on the column, and a faintly colored benzene eluate, but the reverse was the case with the sodium hydroxide-soluble fraction (*C*). The eluate was more colored than the band on the column. The two eluates were combined, concentrated to about 5 ml., and the concentrate used for spotting (*G*).

The bands on the two columns were extruded and extracted with alcohol containing 0.5% acetic acid. The two extracts were combined, concentrated to about 5 ml., and the concentrate used for spotting (*H*). Sennidin A in alcohol, sennidin B in chloroform, and some known anthraquinone (Table II) in benzene were spotted on the same paper. The chromatograms were developed with toluene and sprayed with alcoholic 1 *N* sodium hydroxide. The results are reported in Table II.

Though all the four spots, *D*, *E*, *F*, and *H*(*1*) showed a similar behavior in having R_f 0.00; they differed in the color produced with 1 *N* sodium hydroxide; *E* and *F* developed bright yellow which slowly changed to violet on standing, and *D* and *H*-(*1*) under the identical conditions gave a pink color.

This differentiated the spots *E* and *F* due to bimolecular anthranols from the spots *D* and *H(1)* due to monomolecular anthraquinones. Hence it was clear that while benzene concentrate *G* contained chrysophanol, the spot *H(1)* most probably was due to rhein; the spot *H(2)*, however, did not correspond to any of the aglycones chromatographed.

Chromatography with a second solvent system: lower phase of water:acetone:benzene (2:1:4) gave the following R_f values: chrysophanol, 0.00; rhein, 0.74; benzene solution, 0.00; alcohol solution, (*1*) 0.74, (*2*) 0.89; *C* + *G*, 0.00; *D* + *H*, (*1*) 0.74 and (*2*) 0.89. All the spots were colored pink with 1 *N* sodium hydroxide.

In the above paragraph *H(1)* is evidently due to rhein as indicated by R_f values of rhein and *D* + *H* (*1*). Similarly, comparison of chrysophanol, benzene solution, and *C* + *G* indicated that the second aglycone was chrysophanol.

Identification of Sugars

The aqueous acidic layer (mentioned in *Identification of the New Aglycones*) was cooled, neutralized with sodium hydroxide pellets, and concentrated to dryness using high vacuum. The 1:3 filtered aqueous pyridine extract of the residue was used for spotting on Whatman No. 1 paper. For comparison, a glucose solution and a mixture of the two were spotted simultaneously.

The chromatograms were developed with *n*-butanol:glacial acetic acid:water (4:1:5), by radial chromatographic techniques, and sprayed with Partridge's reagent (5). The average of four R_f values were found to be—glucose, 0.32; aldose from the acid precipitate, 0.30; and a mixture of glucose and the aldose, 0.33. This clearly indicated that the sugar of the glycosides present in the acid precipitate was glucose.

DISCUSSION

In this study the drug was extracted with water instead of alcohol. This not only made the process more economical, but also permitted better separation of the acid precipitate. Since the glucosides present in the drug were thermolabile, all concentrations by heat even under reduced pressure were avoided, and a concentrated extract was obtained by following the procedure of repercolation.

Precipitation of the aqueous extract at different pH, from 1.0 to 7.0, showed that the highest yield of the so-called acid precipitate was obtained at pH 3. This precipitate showed the presence of a laxative principle when tested on albino mice. Tested similarly, the second fraction obtained from the filtrate on addition of calcium chloride and ammonium chloride at pH 7 was inactive, but the third fraction obtained on addition of sufficient alcohol to the mother liquor from the second fraction was again active. A detailed report on the biological assay of the different fraction will be published separately. The fraction II (*C*) contained sennoside A and B, the presence of which is already reported by Stoll (1).

The glucosides present in the acid precipitates could not be crystallized, but they have been shown to be different from sennosides A and B by chromatographic techniques. In addition, the new glycosides have been shown to be those of rhein and chrysophanic acid by cochromatography from two different solvent systems. The sugar part of the glycosides is glucose in both the cases.

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Two Disintegrating Agents *versus* Cornstarch for Compressed Tablets

By G. HECHT† and C. L. HUYCK‡

Cornstarch, Dry-Flo, and Solka-Floc BW 40, were tested for suitability as disintegrating agents. The Dry-Flo and the cornstarch proved favorable for the sodium bicarbonate and the acetylsalicylic acid tablets. It was noted that Solka-Floc BW 40 (due to its fibrous nature) helped to prevent capping of the tablets. Analysis of the disintegration tests of tablets containing Solka-Floc BW 40 and Dry-Flo were statistically different than cornstarch at the 1 per cent probability level.

Cornstarch has been used in compressed tablets as a disintegrant for decades and is still the disintegrant of choice. Dried corn or potato starch is sometimes used with the object of shortening the disintegration time of the tablet, when moisture is detrimental to the stability of the active ingredients (*i.e.*, in aspirin tablets), and in tablets containing medicinals that are incompatible with each other. Even though cornstarch is quite satisfactory, the search continues for faster and more efficient disintegrants. The quicker the release of the medicament after ingestion, the greater the efficiency of the tablet. In this study, two disintegrants are compared with cornstarch and the results are analyzed statistically.

Two compounds were chosen to be tested as tablet disintegrants: Solka Floc BW 40[1] (a purified wood cellulose) and Dry-Flo[2] (a starch ester containing a hydrophobic group). As a control disintegrant, 10% cornstarch was used.

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