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Estimation of *C*-glycosides and *O*-glycosides in cascara (*Rhamnus purshiana* DC., bark) and cascara extract

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An assay process is described which enables the cascarosides, the aloins and the *O*-glycosides of cascara to be estimated separately. The cascarosides are separated from the aloins by partition between water and ethyl acetate; each fraction is then oxidised and hydrolysed by ferric chloride treatment to the free anthraquinones which are determined colorimetrically as aloe-emodin. The *O*-glycosides are determined colorimetrically as emodin after acid hydrolysis. Results show that commercial extracts contain only about half the theoretical amount of *C*-glycosides and about quarter of the *O*-glycosides and there is a significant breakdown of primary glycosides. These results indicate that decomposition occurs during preparation of the official extract. One commercially available extract, which passed current official standards, was shown by the method to be very deficient in activity. In view of the small proportion of *O*-glycosides in commercial extracts a shortened assay process, for cascarosides and aloins only, is described.

CASCARA bark contains the following classes of anthracene derivatives: (*a*) *cascarosides*, which are primary glycosides of barbaloin and chrysaloin and of which about four have been identified; (*b*) *aloins*, barbaloin and chrysaloin, which are *C*-glycosides of aloe-emodin anthrone and chrysophanol anthrone respectively; (*c*) O-*glycosides*, mostly based on emodin and of which about four are present and (*d*) the free *anthraquinones*, aloe-emodin, chrysophanol and emodin (Fairbairn & Simic, 1960). The cascarosides are probably the most important components. In preliminary experiments we have found them more active biologically than the aloins, which is consistent with the fact that the primary glycosides of senna are more active than the secondary ones (Fairbairn, Friedmann & Ryan, 1958). Furthermore they have a sweet taste in contrast to the aloins, and are therefore preferable pharmaceutically. A chemical method of evaluating cascara should therefore estimate the cascarosides separately from the aloins. The free anthraquinones have little purgative activity (Fairbairn, 1949) and should not be included by the assay process. Recently Auterhoff & Sachdev (1962) published a method for estimating the *C*-glycosides and *O*-glycosides of cascara but their method does not estimate the cascarosides separately from the aloins nor does it eliminate the free compounds. We have therefore attempted to devise a chemical method of assay which would estimate the three important classes of anthracene derivatives separately. Bark and extracts conforming to B.P. specifications were examined.

Experimental

EXTRACTION OF THE GLYCOSIDES FROM THE BARK AND EXTRACT

Extraction with water was unsuitable as subsequent treatment with organic solvents led to troublesome emulsions. The use of 70% methanol as extracting solvent prevented this; furthermore 5 to 30% more glycosides were extracted in a given time with this solvent than with water. Some samples of extract dissolved rapidly in the 70% methanol; others were

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much more difficult. We therefore recommend, as a general method, standing in the solvent overnight.

SEPARATION OF THE CASCAROSIDES FROM THE ALOINS

The cascarosides are much more water soluble than the aloins and we found that the partition coefficient of barbaloin, in the system ethyl acetate: water, was 1·0 whereas for cascaroside A it was almost zero. Using an artificial mixture of barbaloin and cascaroside A it was possible to effect 96 to 99% separation by distribution between ethyl acetate and water under suitable conditions. When applied to the more complex mixtures occurring in the crude drug these conditions (slightly modified) were shown to be successful by chromatographic examination of each stage.

ESTIMATION OF THE *O*-GLYCOSIDES

Acid hydrolysis followed by extraction of the liberated aglycones seemed the obvious way of estimating these glycosides. However, the hydrolysis process also converts the cascarosides to aloins, so that it must be applied *after* the separation of these two classes of substance from each other. This separation process unfortunately results in the *O*-glycosides being distributed fairly equally between the ethyl acetate and water layers. Each layer has therefore to be treated with acid and the liberated aglycones removed before the *C*-glycosides present are estimated. Details of how this is effected are given in the method of assay below.

STANDARDS

Since the four known cascarosides have a closely similar structure they are estimated as cascaroside A which we found, by preparative methods, to be present in the largest proportion. Treatment of pure cascaroside A by the ferric chloride treatment detailed below showed that 1 mg of aloe-emodin corresponded to 2·56 mg cascaroside A (an exactly similar result was obtained with cascaroside B). The aloins were estimated as barbaloin (Fairbairn & Simic, 1963) and the *O*-glycosides as emodin monoglucoside a pure sample of which was separated from the bark (Simic, 1961). Another emodin monoglucoside has also been shown to be present in cascara (Schindler, 1946).

RECOMMENDED METHOD OF ASSAY

Transfer about 1 g powdered bark or 0·5 g powdered extract, accurately weighed, to a 100 ml volumetric flask by means of 80 ml of 70% methanol. Allow to stand overnight with occasional shaking; make up to volume with 70% methanol shake well and filter. To 10 ml of the filtrate add 10 ml water and extract 2 to 3 times with 20 ml portions of carbon tetrachloride. Wash the combined carbon tetrachloride extracts with 10 ml of water, reject the carbon tetrachloride layer and return the washings to the aqueous layer. Extract the combined aqueous layers with water-saturated ethyl acetate (4 × 30 ml for the bark and 5 × 60 ml for the extract) and reserve both layers for further work.

ALOINS

Evaporate the ethyl acetate layer to small volume and transfer to a 100 ml conical flask and evaporate to dryness. Dissolve the residue in 0·3 to 0·5 ml methanol, add 10 ml N hydrochloric acid, attach a short air condenser to the flask and heat in a boiling water-bath for 15 min. Cool, transfer to a separator and rinse the flask with 2 to 3 ml N sodium hydroxide followed by 2 to 3 ml water. Shake the combined aqueous layers with 20 ml carbon tetrachloride, allow the layers to separate and draw off the lower carbon tetrachloride layer. Run the intermediate emulsified layer into a small beaker, dissolve in about 1 ml N sodium hydroxide and return to the aqueous layer. Repeat the extraction of the aqueous layer in a similar manner with two more portions of 20 ml carbon tetrachloride. Filter the aqueous layer into a 25 ml volumetric flask; wash the carbon tetrachloride layer with small quantities of water and pass through the filter and make up to volume. (Reserve the carbon tetrachloride layer for assay of the *O*-glycosides). To a suitable aliquot of the filtrate (20 ml for the bark; 5 or 10 ml for the extract but in each case made up to 20 ml with water) add 1·2 g anhydrous ferric chloride, 12 ml hydrochloric acid B.P. and heat in a boiling water-bath, under reflux, for 4 hr. Cool, extract with 3 × 20 ml portions of carbon tetrachloride and wash the combined carbon tetrachloride extracts with 2 × 10 ml water. Reject the washings. Extract the carbon tetrachloride layer with 10,5 and 5 ml N sodium hydroxide; heat the combined alkaline extracts in a boiling water-bath for 5 min (to drive off traces of carbon tetrachloride), cool and make up to 25 ml. Determine the extinction of this solution at 500 mμ, within 1 hr, and estimate the concentration of aloe-emodin from the E (1% 1 cm) value of 320 or from a suitable calibration curve. Calculate the percentage of aloins present as barbaloin from the fact that 1 mg aloe-emodin is equivalent to 1·61 mg $C_{21}H_{22}O_9 \cdot H_2O$.

CASCAROSIDES

To the aqueous layer obtained by the preliminary treatment, add 3 ml hydrochloric acid, and heat in a conical flask to which a short air condenser is attached in a boiling water-bath for 15 min. Cool, extract with 3 × 20 ml carbon tetrachloride and treat the intermediate emulsified layer as previously described. Filter the aqueous layer into a 50 ml volumetric flask. Wash the combined carbon tetrachloride layers with small quantities of water and pass them through the filter and make up to volume. (Reserve the carbon tetrachloride layer for assay of *O*-glycosides). To 10 ml of the filtrate add 0·6 g anhydrous ferric chloride 6 ml hydrochloric acid B.P. and heat in a boiling water-bath, under reflux, for 4 hr. Continue as for ALOINS beginning: Cool, extract with 3 × 20 ml carbon tetrachloride. . . . Calculate the percentage of cascarosides as cascaroside A. 1 mg aloe-emodin is equivalent to 2·56 mg cascaroside A.

O-GLYCOSIDES

Combine the carbon tetrachloride extracts from the "aloins" and "cascarosides" stages; extract with 10,5 and 5 ml N sodium hydroxide.

Heat the combined extracts in a boiling water-bath for 5 min. Cool and make up to 25 or 50 ml according to intensity of colour. Determine the extinction of this solution at 510 mμ, within 1 hr, and estimate the concentration of emodin from the E (1%, 1 cm) value of 330 or from a suitable calibration curve. Calculate the percentage of *O*-glycosides as emodin monoglucoside. 1 mg emodin is equivalent to 1·60 mg emodin monoglucoside.

Results

Replicate assays on one sample (A) of extract were made using several separate weighings and the standard deviation of the results calculated. This indicates a reproducibility for a single assay (P = 0·95) of about $\pm$ 3·6% for the cascarosides; $\pm$ 1% for the aloins and $\pm$6% for the *O*-glycosides. Several other commercial extracts and samples of bark were also assayed in duplicate and the results recorded in Table 1.

TABLE 1. ANALYSES OF COMMERCIAL SAMPLES OF CASCARA BARK AND EXTRACTS CONFORMING TO B.P. SPECIFICATIONS. RESULTS ARE EXPRESSED AS g/100 g AIR-DRY SAMPLE (1 g EXTRACT SHOULD BE EQUIVALENT TO 4 g BARK)
Each figure (except for extract A) is the average of two assays.

Sample	*C*-Glycosides			*O*-Glycosides (as emodin glucoside)
	Cascarosides (as cascaroside A)	Aloins (as barbaloin)	Total (as barbaloin)	
Bark				
B_1 1954	6·83	0·42	4·72	1·22
B_2 1957	5·22	0·80	4·08	0·98
B_3 1962	6·76	0·44	4·69	0·74
B_4 1962	7·67	0·69	5·51	0·91
B_5 1962	6·60	0·68	4·75	0·72
Extract				
A 1963	9·72	2·98	9·11	1·11
(Means of 6 assays)	(s. d. = 0·175)	(s. d. = 0·016)		(s. d. = 0·035)
B 1963	9·32	7·80	13·40	0·44
C 1962	6·93	5·11	9·47	0·90
D 1955	7·68	3·50	8·33	0·53
E 1955	5·40	6·77	10·17	1·0
F 1954	1·20	1·59	2·26	0·21

Discussion

OFFICIAL PREPARATIONS OF CASCARA

The results in Table 1 indicate two deficiencies in the official methods of preparing dry extract of cascara, 1 g of which should correspond approximately to 4 g of bark. For the 5 samples of bark examined the average content of *C*-glycosides (calculated as barbaloin) is 4·75%, and for *O*-glycosides is 0·90%. For the 5 samples of extract (A to E) the corresponding figures are 10·10% and 0·80%. Clearly there has been a significant loss of both classes of constituents in preparing the official extract. Secondly the proportion of cascarosides to aloins is much lower in the extract than in the bark. This indicates that decomposition has taken place during evaporation of the bulky aqueous percolate and this suggestion is confirmed by the low amount of the labile *O*-glycosides in the extract.

In spite of these deficiencies the final product contains a significant amount of the relatively thermostable aloins which would account for the

fact that cascara tablets have been accepted for a long time as an effective purgative. This is in contrast with the former official preparations of senna whose less stable *O*-glycosides largely disappear during preparation and storage of the galenicals (Fairbairn & Michaels, 1951). The results for extract F however show that poor quality extracts occasionally appear on the market. Since the proportion of individual components is similar to the other extracts the figures recorded suggest that this extract contains a small proportion of genuine extract of cascara to which has been added about 4 times the weight of water soluble diluent. The final "extract" passes all the B.P. tests for identity and purity, etc., but is obviously deficient in potency. This deficiency would be readily detected by the chemical assay process described in this paper.

SHORTENED ASSAY PROCESS

The results in Table 1 show that the *O*-glycosides only represent about 10% of the total anthracene glycosides of the extract. In view of this, and also the fact that their potency is not likely to differ markedly from the other glycosides, it is probably unnecessary to estimate them separately. If they are included in the other classes of compounds the assay process can be considerably shortened. The removal of the free compounds and separation of the glycosides into ethyl acetate soluble and water soluble fractions are carried out exactly as detailed for the assay of the extract. The *ethyl acetate fraction* is evaporated to dryness, dissolved in water to 25 or 50 ml, as described; 10 ml is heated with 0·6 g anhydrous ferric chloride and 6 ml hydrochloric acid B.P. under reflux for 4 hr. After cooling the hydrolysate is extracted with carbon tetrachloride, the latter extracted with N sodium hydroxide, made up to volume and, after colorimetric measurement, the amount of aloins calculated as already described. The *aqueous fraction* is diluted to 50 ml, and to 10 ml is added 0·6 g anhydrous ferric chloride 6 ml hydrochloric acid B.P. and the above process repeated. After colorimetric estimation the amount of cascarosides present is calculated as previously described. Besides reducing the time of assay this modified process also eliminates the troublesome middle layers which arise when the longer assay process is used. Experiments with some of the extracts analysed by the longer process showed that the normal glycosides were fairly evenly distributed between the cascarosides and the aloins when the shortened process was used.

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