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A new dry extract of cascara (*Rhamnus purshiana* D.C. bark)

J. W. FAIRBAIRN AND S. SIMIC

*Department of Pharmacognosy, The School of Pharmacy, University of London, Brunswick Square, London, W.C.*1, *U.K.*

The B.P. method of preparing a dry extract of cascara leads to significant breakdown of *O*-glycoside links in the active compounds. This is shown to be due to enzyme activity during percolation. A dry extract, containing most of the active glycosides originally present in the bark with no change in glycosylation status, can be prepared using boiling water as extracting solvent. A modification of the chemical assay process is recommended as well as the use of an official standardized extract with *upper* and lower limits for glycoside content.

Analysis of samples of cascara bark and of dry extract of cascara B.P. (Fairbairn & Simic, 1964) showed that the extracts contained only about half of the theoretical amount of anthraquinone glycosides and that there had been significant breakdown of *O*-glycosides. These conclusions were confirmed (*Analyst*, 1968). As Fairbairn & Simic found ethanol (70%) to be a good extracting solvent, an extract using this solvent has been made and compared with one using the cold water extraction method of the B.P. 1968 and one made with boiling water because enzyme action during extraction proved to be influencing the yield of the constituents.

EXPERIMENTAL AND RESULTS

Powdered bark (100 g) was percolated with 70% ethanol to give 500 ml of percolate which was evaporated to dryness in a vacuum. A further 100 g of the same bark sample was percolated with water to exhaustion (1310 ml percolate) and evaporated to dryness (B.P. 1968). The original bark, the percolates and the final extracts were analysed (Fairbairn & Simic, 1964) and the results recorded in Table 1. Unexpectedly, the main destruction of the *O*-glycoside link (conversion of cascarosides to aloins as well as loss of *O*-glycosides) was found to occur during percolation, possibly because glucosidases of the bark pass into the percolate and bring about hydrolysis during the 2 to 3 day process.

Enzymatic studies

Ten g of the same bark sample was percolated with cold water (100 ml) and to the percolate increasing quantities of ammonium sulphate were added and dissolved. The precipitate formed after each addition was centrifuged, re-dissolved in water and dialysed. The dialysed solution was extracted with ethyl acetate to remove traces of anthraquinone compounds and the glucosidase activity assessed by determining the hydrolytic effect on pure cascarosides. The precipitate formed at 30% saturation with ammonium sulphate was the most active and a solution of it was prepared and 0·5 ml quantities were mixed separately with known quantities of cascaroside A and cascaroside B and allowed to stand at room temperature (20°) and pH about 5. Samples were taken at intervals, diluted with water and extracted

Table 1. *Comparative analyses of dried extracts from the same sample of Bark* (100 g), *made by the B.P. 1968 process, percolation with 70% ethanol and boiling water.* Glycosides calculated as cascaroside A; each figure is the mean of two assays

Bark	Cascarosides	Aloins	*O*-glycosides: Ethyl acetate insoluble	*O*-glycosides: Ethyl acetate soluble	Total glycosides	Free compounds (as emodin)
100 g	4·46	1·86	0·43	0·99	7·74	0·83
B.P. Method						
Percolate 1310 ml	2·24	3·39	0·17	0·21	6·01	0·23
Dry extract 28·5 g	2·16	3·09	0·14	0·17	5·56*	0·22
70% Ethanol						
Percolate 500 ml	3·56	2·96	0·28	0·56	7·36	1·07
Dry extract 31·2 g	3·40	2·91	0·30	0·48	7·09†	1·07
Boiling water						
Decoction 954 ml	5·08	1·21	0·35	1·62	8·26	0·19
Dry extract 30·8 g	4·80	1·30	0·27	1·80	8·17‡	0·17
Assay of bark (new method)	4·98	1·55	0·33	1·79	8·65	0·20

* Equivalent to 19·5%, † 22·7%, ‡ 26·5% of the dry extract.

with ethyl acetate to remove barbaloin and other hydrolytic products of the cascarosides. The amount of unchanged cascaroside was determined from the absorbance at 325–327 nm, a diluted solution of the enzyme being used as a blank and boiled enzyme solution, similarly treated, as a control.

The results in Table 2 show that there was significant hydrolysis of cascaroside B compared with the control but little hydrolysis of cascaroside A. This specificity was checked by repeated experiments and also by TLC examination of the enzyme treated glycosides on Silica gel G plates using methyl ethyl ketone–ethyl acetate–methanol–water (10:5:2:2). As a further control, aqueous solutions of cascarosides A and B at pH about 5 were stored at room temperature but no significant hydrolysis took place after 3 days. Similarly, treatment with boiling water for 15 to 30 min produced little hydrolysis, indicating that the glycosides are not so thermolabile as previously thought (*Analyst*, 1968).

Table 2. *Effect of an enzyme preparation and of other conditions on the stability of the cascarosides.* (t_0 = at zero time)

Conditions	Cascaroside A: Amount	Cascaroside A: Loss	Cascaroside B: Amount	Cascaroside B: Loss
Enzyme t_0	3·14 mg		2·93 mg	
Enzyme t_0 + 3 days	2·93 mg	6·7%	2·32 mg	20·8%
Enzyme boiled t_0 + 3 days	3·20	0%	2·74	6·5%
Water alone, t_0 + 3 days	—	—	2·82	3·7%
Boiling water, 15 min, then cooled	—	—	2·93	0·0%

Boiling water extract

As boiling water destroyed enzyme activity and had little effect on the cascarosides, we prepared an extract by adding to 100 ml of boiling water 10 g of the same sample of bark slowly with stirring. After being boiled for a further 5 min, the decoction was cooled, made up to 100 ml, filtered and a suitable aliquot evaporated to dryness

in vacuo and then analysed. From the weight of the decoction and the weight per ml of the filtrate the water-insoluble fraction of the bark was estimated to be 4·6 ml of the 100 ml of decoction and this was used to correct the analysis results in Table 1 which have also been adjusted to give figures for 100 g of bark for comparison with other figures in the Table. Much less breakdown of *O*-glycosides occurred than with the other extracts and the overall yield of glycosides is practically 100%.

Modified assay procedure

As the results in Table 1 also show that the boiling water extract contains more glycosides than the original bark assayed using the 70% ethanol extract, the sample of bark was re-assayed by the previous *Analyst* (1968) method modified by adding 1 g of powdered bark to 100 ml of boiling water and boiling for 5 min, cooling and making up to 100 ml, 10 ml of the filtrate being used for assay. The results are recorded in Table 1.

DISCUSSION

A potent and standardized dry extract of cascara

Cold percolation with water, or 70% ethanol, extracts sufficient enzymes from the bark to produce significant destruction of the *O*-glycoside link during percolation. This seems mainly responsible for the diminished yield of total glycosides and the conversion of cascarosides to aloins in official dry extracts. Boiling water prevents these losses and changes and a potent extract containing nearly all the anthraquinone glycosides originally present in the bark, with only slight changes in their glycosylation status, has been prepared. The U.S.P. XIV (1950) used boiling water for the preparation of cascara sagrada extract and the B.P. 1968 uses boiling water for the preparation of cascara elixir but not for the dry or liquid extract.

Published figures (Fairbairn & Simic, 1964; *Analyst*, 1968) show that the glycoside content of 10 samples of bark varied from 8 to 10·3%; the amount of boiling water extractive would also be expected to vary significantly so that the percentage glycosides in the final extract would also vary widely, therefore we suggest that official extracts should be standardized chemically with both *upper* and lower limits of glycosides, together with a minimum proportion of cascarosides.

Modification of the chemical assay process

The use of boiling water gives a slightly higher yield of glycosides in the assay process and is quicker and more convenient and we therefore recommend its adoption in the pharmacopoeial monograph.

The "shortened" assay process recommended in the *Analyst* Report (1968) estimates only the "cascarosides" (water soluble glycosides) and the "aloins" (ethyl acetate soluble glycosides) but the figures in Table 1 are based on the "long" assay process (Fairbairn & Simic, 1964). However, as they are all calculated as cascaroside A, they can be readily converted into "cascarosides" (water soluble glycosides) by adding together the figures in column 2 and 4, and into "aloins" (ethyl acetate soluble glycosides) by adding together the figures in columns 3 and 5.

REFERENCES

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