VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART XI. FURTHER WORK ON THE ALOIN-LIKE SUBSTANCE OF *Rhamnus purshiana* DC.

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The aloin-like substance formerly referred to as Compound A_1, has been resolved into four closely allied anthraquinone derivatives by paper chromatographic and countercurrent techniques. Two of these substances have been isolated in pure form and their general properties, melting point, optical rotation, R_F values and ultra-violet light absorption curves are recorded. Treatment with ferric chloride yields aloe-emodin from both, and each on mild hydrolysis, produces barbaloin. The names Cascaroside A and Cascaroside B are proposed. Preliminary work on the other two components has shown that they are based on a compound similar to barbaloin but a derivative of chrysophanol instead of aloe-emodin; the name chrysaloin is suggested for this substance.

IT was shown previously[1] that cascara bark (*Rhamnus purshiana* bark) contains an aloin-like substance which was named Compound A. Although this substance always gave one spot on the paper chromatographic system used, we were not satisfied it was a pure substance, mainly because of the uncertain melting point and the fact that the extinction values at the peaks of the ultra-violet light absorption curve varied from batch to batch. The proportion of aloe-emodin and chrysophanol produced by ferric chloride treatment varies from batch to batch, an observation which is also consistent with the supposition that Compound A is a mixture.

EXPERIMENTAL

Experience had shown that prolonged exposure to solvent systems containing acids led to changes in the compounds. Thus the methods of Adamis and Pawlaczyk[2] for *Rhamnus frangula* and Awe, Auterhoff and Wachsmuth-Melm[3] for aloe were attempted but not used extensively because of the presence of acetic acid. Also, the separation effected was not much better than that occurring when our previous systems were used.

Paper Chromatographic Systems

Belaart[4] used the system *n*-butanol: ethanol: water (5 : 1 : 4) for investigating *Rheum* species. Using this system, the upper layer as running solvent and Whatman No. 1 paper, by ascending technique, the original Compound A showed three spots on development. The upper one ($R_F = 0{\cdot}43$) on elution and ferric chloride treatment[1] yielded chrysophanol only: the two lower ones ($R_F = 0{\cdot}31$ and $0{\cdot}25$) both yielded aloe-emodin only.

Further experimenting led to the discovery of another useful system; ethylmethyl ketone: methanol: water (20 : 1 : 5). When used in the same conditions as the previous one the original Compound A was resolved into two upper spots ($R_F = 0{\cdot}50$ and $0{\cdot}44$) both yielding chrysophanol only

on ferric chloride treatment and two lower spots ($R_F = 0{\cdot}32$ and $0{\cdot}23$) both yielding aloe-emodin only on ferric chloride treatment. Subsequent work, to be described later, showed that the two substances with low R_F value both yielded barbaloin on mild hydrolysis. In similar circumstances the two substances of higher R_F value yielded a compound corresponding to barbaloin but based on chrysophanol instead of aloe-emodin. We propose to call this second aloin-like substance *chrysaloin*. Baumgartner and Leupin[5] state they have isolated barbaloin from cascara bark; we have confirmed this and have shown that chrysaloin also occurs as such in the bark.

To confirm that these four compounds were single substances we decided to apply countercurrent methods.

Countercurrent Systems

Since we had to work with compounds whose physical and chemical properties were largely unknown it was necessary to work on empirical lines in devising suitable solvent systems. Our previous experience with the use of selective solvents and paper chromatographic systems led us to choose the following for preliminary investigation; acetone, benzene, ethanol, ether, ethyl acetate, isopropanol, methanol and water. Various mixtures containing two or three of these solvents were prepared so that two layers were formed, the volumes of the two layers were not too dissimilar, and, on shaking and allowing to settle the layers separated readily. Mixtures which passed these tests were then used on strip chromatograms in boiling tubes. Those systems which resolved Compound A into two to four spots (none resolved it into more than four spots) were then selected for further testing in a small hand-operated countercurrent machine containing Craig tubes. On this basis the following three systems were discovered. Ether : isopropanol : water (2 : 1 : 2), Ethylmethyl ketone : water (4 : 3), n-butanol : ethanol : water (5 : 1 : 4). Two of the successful systems are almost similar to those used for paper chromatographic work.

This additional work did not reveal any further resolution of Compound A into more than four anthraquinone compounds but it did show that certain compounds fluorescing blue in ultra-violet light had R_F values identical with those of some of the four compounds. Furthermore, when an extract of the bark was used as starting material, a large number of anthraquinone compounds was revealed, some of which had R_F values similar to the compounds we were extracting. These were all successfully separated.

Use of Selective Solvents

Much of the previous work[1] was confirmed but in addition it was shown that ethyl acetate was useful in separating free anthraquinones, barbaloin, chrysaloin and other impurities from the fractions rich in "Compound A."

Isolation of Two New Compounds

A methanolic extract of the bark was prepared as previously described[1]. 100 g. of this extract was dissolved in 250 ml. of methanol and slowly

poured, with vigorous stirring, into 5 litres of ethyl acetate. The ochre-coloured precipitate, weighing about 80 g., was collected, dried, and extracted with four successive quantities of 800 ml. of boiling isopropanol. Each hot extract was filtered immediately, the filtrates combined and allowed to cool in a refrigerator. The yellow precipitate (about 40 g.) was collected, dissolved in 400 ml. water and exhaustively extracted with ethyl acetate in a continuous liquid:liquid extractor. This process removed practically all the barbaloin, chrysaloin, and certain emodin glycosides from the aqueous solution. The latter was evaporated to dryness at a low temperature in a rotating vacuum evaporator, leaving a yellow residue weighing about 30 g. Batches of 15 g. of this residue were dissolved in 28 ml. of the lower phase of the system ethylmethyl ketone: water (4:3), making a volume of about 36 ml. 18 ml. each were introduced into the first two tubes of an automatic countercurrent apparatus. Using 20 ml. portions of upper and lower layers of the above solvent system sixty transfers were effected. Paper chromatographic examination of each of the resulting 120 layers showed that the barbaloin and chrysaloin compounds were contained in the lower aqueous layers as follows. Tube 1, brown pigments mainly; tubes 2 to 5, brown pigments plus the bulk of the barbaloin compounds and some chrysaloin compounds; tubes 6 to 9, mainly chrysaloin compounds with traces of barbaloin compounds; tubes 10 to 16, mainly chrysaloin compounds with traces of certain compounds fluorescing blue in ultra-violet light. Other anthraquinone compounds were distributed elsewhere and suitable layers were retained for future work. The contents of tubes 2 to 5 (lower layers) were combined and evaporated to dryness and the residue (about 6 g.) dissolved in 24 ml. of the lower phase of the system *n*-butanol: ethanol: water (5:1:4). This solution was treated in the automatic countercurrent apparatus as before, using 20 ml. portions of the upper and lower phases of the solvent system just mentioned, and thirty transfers. Paper chromatographic examination of each of the layers showed that the barbaloin compounds had been separated from traces of chrysaloin compounds. The contents of the appropriate tubes containing barbaloin compounds were evaporated to dryness *in vacuo* and the solid residue extracted with hot isopropanol. On cooling a buff yellow precipitate was formed. This was separated, dried and dissolved in about 10 ml. of methanol and the solution slowly poured into 8 volumes of ethylmethyl ketone. After allowing the precipitate to settle the supernatant liquid was decanted, the precipitate washed with ethylmethyl ketone and allowed to dry in a vacuum desiccator. This precipitate contained only the two barbaloin compounds referred to earlier. These two were separated by band chromatography, using the ethylmethyl ketone system, and purified by elution with methanol and precipitation from ethylmethyl ketone as already described.

The entire process was repeated so that two independent batches of the barbaloin compounds were prepared. A third batch was prepared from a sample of Compound A prepared in 1958 by the method previously described[1]. The properties of the three batches were sufficiently

consistent to conclude that the two barbaloin compounds were pure substances and we propose to call them *Cascaroside A* and *Cascaroside B*.

Properties of Cascaroside A and B

Some of the physical and chemical properties of the two cascarosides are shown in Table I, and the ultra-violet light curve of cascaroside A is shown in Figure 1.

Mild Hydrolysis

Both cascaroside A and B, on heating in N hydrochloric acid at 70° for 2 hours broke down into barbaloin. This was proved by comparison

TABLE I

PROPERTIES OF CASCAROSIDES A AND B

(Note: The letters (*a*) (*b*) and (*c*) refer to separate batches of these compounds)

Properties	Cascaroside A	Cascaroside B
1. General	Buff-coloured powder; taste, sweet followed by a slight bitterness. Very soluble in water: Soluble in methanol, ethanol. Almost insoluble in acetone, chloroform and ether.	
2. R_F values n-Butanol system Ethylmethyl ketone system	0·31 0·32	0·25 0·23
3. Ultra-violet light absorption curves Peaks (mμ) E(1 per cent, 1 cm.)	267 : 295 : 323·5 (*a*) 112 157 135 (*b*) (i) 108 153 131 (*c*) 118 163 141	267 : 294 : 326 (*a*) 106 146 129 (*b*) (i) 113 157 138 (*c*) 112 158 141
4. Melting point Kofler	(*a*) 180–181° (*b*) (i) 168–169° *(ii) (*c*) 189–190°	(*a*) 165–167° (*b*) (i) 165–167° *(ii) (*c*) 181–182°
5. Optical rotation $[\alpha]_D^{19}$	(*a*) −40° (C=0·8 Ethanol) (*b*) (i) −28° (C=1·7 Water)	(*a*) −110° (C=0·8 Ethanol) (*b*) (ii) −76° (C=0·9 Water)

* After further purification by re-chromatographing and re-precipitating.

with authentic barbaloin using paper chromatographic examination, ultra-violet light curves, melting point of the isolated hydrolytic product and general physical and chemical properties. The following two procedures were used. 1. About 60 mg. of cascaroside was dissolved in 10 ml. N hydrochloric acid and kept at 70° for 2 hours. The solution was cooled and extracted with three portions of carbon tetrachloride which were rejected. The aqueous phase was then extracted with 20, 10, 10 and 10 ml. portions of ethyl acetate, the ethyl acetate extracts combined and stored over anhydrous sodium sulphate and then filtered. The filtrate was evaporated to dryness at low temperature and the brownish yellow residue stored in a vacuum desiccator (aluminium oxide) overnight. The dry residue was dissolved in 0·4 ml. warm anhydrous methanol and 1·5 ml. of carbon tetrachloride slowly added, warming the solution to redissolve any precipitate that formed. The slightly opalescent solution was allowed to cool slowly when fine yellow crystals were formed. Microscopic examination showed them to consist of well formed needles of low birefringence,

which were identical with those of barbaloin prepared in a similar manner. The melting point (Kofler) was 143° (barbaloin, 148°). The crystals were also used for paper chromatographic examination and ultra-violet light absorption curves as described in the next paragraph. 2. About 20 mg. cascaroside, dried *in vacuo* (magnesium perchlorate) at 80–100° to constant weight, was dissolved in about 10 ml. N hydrochloric acid and heated at 70° for 2 hours. The solution was cooled and made up to volume. Paper chromatographic examination showed the presence of a major spot having the same R_F value and other characteristics as pure barbaloin. The two paper chromatographic systems already referred to

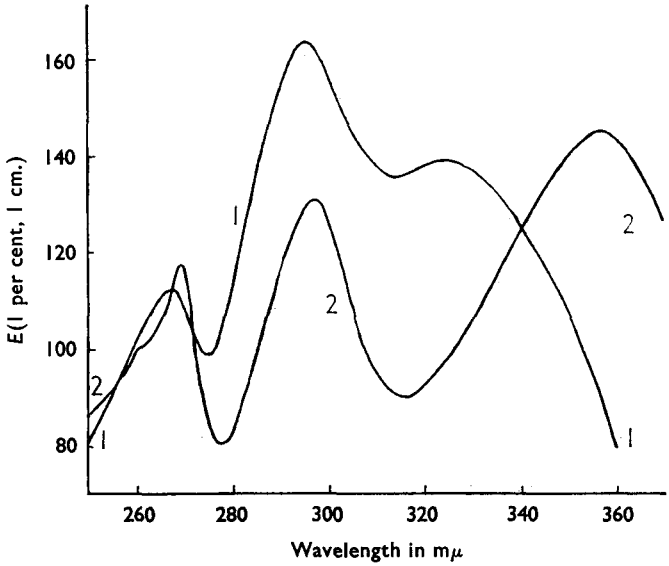

FIG. 1. Ultra-violet light absorption spectra.
1. Cascaroside A.
2. Cascaroside A after hydrolysis in N HCl at 70°.

were used and also water : acetone : benzene (2 : 1 : 4) and n-propanol : ethyl acetate : water (6 : 1 : 3). In all four systems the major spot behaved exactly as a sample of pure barbaloin prepared by the method of Hay and Haynes.[7] Traces of other anthraquinone compounds were seen but these had the same R_F values and characteristics as traces of impurities formed when pure barbaloin was treated with N hydrochloric acid in identical conditions. Spectrophotometric examination of the treated barbaloin showed that there had been no significant alteration in the E(1 per cent, 1 cm.) values taken at the peaks of the ultra-violet light absorption curve. This indicates that the amount of impurities formed is insignificant. The ultra-violet light absorption curve of the hydrolysed cascaroside was therefore determined and compared with that before hydrolysis (See Fig. 1). It will be seen that hydrolysis has produced significant changes and that the new curve has peaks exactly the same as those of barbaloin,

namely at 269, 296 and 354 mμ. The amount of barbaloin in the hydrolysate was calculated using the E(1 per cent, 1 cm.) values given by Lister and Pride[11] and from these figures the amount of barbaloin produced by 1 g. of cascaroside was calculated. The following figures were obtained in two experiments.

		269 mμ	296 mμ	354 mμ
(*a*)	1st experiment	0·550 g.	0·547 g.	0·547 g.
(*b*)	2nd experiment	0·549 g.	0·544 g.	0·559 g.

These results also show that the ratio of the peaks of the curve of the hydrolysed product is identical with that for pure barbaloin.

Discussion

The resolution of our original Compound A into two major components, one based on barbaloin (a derivative of aloe-emodin) and the other on chrysaloin (a derivative of chrysophanol) explains the fact previously reported that Compound A on treatment with ferric chloride yields aloe-emodin and chrysophanol. We have found that these major components vary in solubility in different solvents and this would explain why different batches of Compound A yielded varying proportions of aloe-emodin and chrysophanol.

Each of the major components appeared to consist of two substances and the properties of those from the barbaloin component are shown in Table I. The two substances are clearly very similar and it is probable that they are isomers. The evidence that they are two entities is as follows.

Behaviour in four paper chromatographic systems. At every stage in the extraction procedure, the fractions always showed the same two spots each with characteristic R_F values and slightly different shades of ochre to brownish red in ultra-violet light using the two chromatographic systems already referred to. Two other systems were also used to confirm the results; n-butanol:acetic acid:water (4:1:5), and n-propanol:ethyl acetate:water (6:1:3). In both additional systems two spots always appeared. When isolated in pure form and chromatographed separately or as mixtures the same two spots always appeared. When one substance contained traces of the other, there was always one large spot with a small faint one, corresponding to the proportions present.

Melting point. Each of the three batches mentioned in Table I varied in purity as judged by the melting point, but in all instances the melting point of Cascaroside A was about 10° higher than that of Cascaroside B.

Optical rotation. This is the most decisive evidence although the determination in ethanol was a little uncertain because of a darkening of the compounds when dissolved. However, both sets of figures for optical rotation show a marked difference between A and B.

Cascarosides A and B yield barbaloin on mild hydrolysis. Such a breakdown is likely to occur in the crude drug and its extracts and would explain the reported isolation of barbaloin from cascara[5]. Barbaloin has been shown to consist of aloe-emodin anthrone linked to a sugar group

by a direct carbon to carbon link[6,7]. It was the first -C-C-glycosyl compound to be found in nature and several others have since been reported[8,9]. In cascara a further four seem to be present and future research may well show that such compounds are quite common. As they are not true glycosides, yet consist of a sugar group plus an aglycone, some distinctive name should be devised for this type of natural compound.

The techniques described in this paper have revealed the presence of a number of anthraquinone compounds in cascara. Investigations to date suggest that they fall into three series, those based on aloe-emodin; mainly -C-C-glycosyl compounds; those based on chrysophanol, mainly -C-C-glycosyl compounds; and those based on emodin, mainly "easily split" glycosides.

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After Mr. Simic presented the paper there was a DISCUSSION. The following points were made.

The cascarosides had recently been shown to be true glycosides which on hydrolysis gave the same sugar. The ultra-violet curves were obtained from solutions of pH 7. The pharmacological activity of the two compounds had yet to be assessed.