VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART IX. AN ALOIN-LIKE SUBSTANCE IN *Rhamnus purshiana* DC.

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Received June 1, 1958

A new kind of anthraquinone compound has been isolated from cascara bark (*Rhamnus purshiana*). Unlike the normal glycosides it cannot be hydrolysed by heating in acid solution, but on heating in acidified ferric chloride solution aloe-emodin and a second anthraquinone, possibly chrysophanol, are produced. Some of the physical properties are described including the ultra-violet light absorption curve which shows peaks at 268, 296 and 325 mμ. The relationship of this compound to casanthranol and to the chemical assay and biological activity of cascara is discussed.

WE have already drawn attention to the presence of an aloin-like substance in cascara bark (*Rhamnus purshiana*) and its official Extract[1] and to the paper chromatographic technique used in its detection[2]. This chromatographic method also revealed the presence of two other anthraquinone compounds and for convenience we have called the aloin-like substance, which has the highest R_F value, Compound A; the substance with the next highest R_F value, Compound B; and the third substance Compound C. In the first part of this present paper we describe the isolation and some of the properties of Compound A and later we discuss the relationship of this discovery to previous work on the active principles of cascara.

THE ISOLATION OF COMPOUND A

Several methods have been published for extracting pure glycosides from cascara and we decided to follow these methods in the first place. If pure compounds could be obtained by these means their behaviour on the chromatogram could be compared with our previous results and it was hoped that one of these pure compounds would be identical with our Compound A. Both Schindler[3] and Mühlemann[4] (who worked on the related frangula bark) acetylated a suitable extract of the bark, extracted the acetylated glycosides with benzene and fractionated on silicic acid columns. We found these methods extremely tedious and in no instance did we obtain a pure compound. Chromatographic examination of the final preparations showed that Compound A was present but was admixed with impurities. We suspect that these methods lead to considerable losses due to extensive manipulation and that the original glycosides are altered owing to prolonged exposure to adverse conditions. Sipple, King and Beal[5] used a method which did not involve acetylation; this method was also laborious and yielded a reddish brown material which was a mixture of glycosides together with various impurities. Lee and Berger[6] separated a yellow substance (casanthranol) by fractional

precipitation of a methanolic extract of cascara with acetone; they further purified the precipitate by crystallising from hot *iso*propanol. We found their method a considerable improvement on the others but the final yellow substance obtained, though rich in Compound A, was still contaminated with another anthraquinone compound together with several impurities. However, as this method was the most successful we had tried we decided to use it as a basis for further work.

Since the yellow mixture just referred to could be separated into its components by paper chromatography it seemed obvious that some form of countercurrent technique or of column chromatography on cellulose would effect separation on a larger scale. Several attempts were made to do this, using simple apparatus, but as more efficient facilities were not available we decided to fall back on the older method of fractionation by selective solvents. This decision was also influenced by the discovery we made during the preliminary work that when the yellow substance was distributed in the system nitromethane/water, most of the impurities passed into the nitromethane, while Compound A remained in the aqueous phase. However, before proceeding to this next stage, we decided to investigate some of the physical properties of Compound A by isolating a few mg. by paper chromatography and examining them. This work showed that Compound A was stable in water at 60–70° for several hours, so that it was possible to extract an aqueous solution with nitromethane in a continuous liquid/liquid extractor without decomposing the compound. We decided nevertheless to minimise the time of exposure of the aqueous phase to high temperature in the liquid/liquid extractor, by preliminary extraction with chloroform and by the use of partial vacuum.

Method Used for the Isolation of Compound A

About 1 kilo of cascara bark in No. 20 powder, was extracted to exhaustion with chloroform, to remove free anthraquinones, pigments, fats, etc. The exhausted marc was oven-dried at 70° and then percolated with anhydrous methanol under anhydrous conditions until the percolate was a pale straw colour. The percolate was evaporated to dryness under reduced pressure and the solid residue dried in a vacuum desiccator. It was then extracted with about 500 ml. of boiling *iso*propanol and the hot solution immediately filtered on a Buchner filter. On cooling the filtrate a bulky brownish yellow precipitate formed. By making several extractions with similar quantities of *iso*propanol, about 30 g. of precipitate was obtained.

The precipitate was dissolved in water (10 volumes) and transferred to a suitable liquid/liquid extractor and extracted with chloroform till the liquid syphoning over was colourless. The chloroform was replaced with nitromethane and extraction continued under reduced pressure, until the nitromethane syphoning over was colourless; this process took about 16–20 hours. After this, extraction was continued without the use of vacuum. At this higher temperature further quantities of yellow impurities were removed from the aqueous phase. Extraction was again continued until the nitromethane syphoning over was colourless. The

aqueous layer was evaporated to dryness under reduced pressure and the solid residue stored in a vacuum desiccator to remove traces of moisture.

The dried powder was extracted with several lots of boiling *iso*propanol; on cooling a bright yellow precipitate was formed. This was refluxed with dry acetone for about 15 minutes to remove traces of impurity which imparted a yellow turbidity to the acetone. The process was repeated with further volumes of acetone till the latter was colourless and chromatographic examination of the yellow precipitate showed only one spot corresponding to Compound A. The precipitate was separated and dried over P_2O_5. The yield was about 8 g. Microscopical examination showed that the substance was a fine granular powder and though many attempts were made to obtain crystals, using various solvents, we have not so far succeeded in doing so. The ultra-violet spectrum of this batch was qualitatively identical with others made by slightly different processes and also with two quantities of about 5 mg. which had been prepared by band chromatography, using the paper chromatographic method previously described[2], but the extinction values at the peaks varied quantitatively. This variation would be consistent with the presence of traces of "inert" impurities such as solvent of crystallisation or material whose ultra-violet spectrum is widely removed from that of Compound A. It is unlikely that such impurities would be anthraquinone compounds. The properties described in the following section are those common to all batches so far isolated.

Properties of Compound A

General

Compound A is a yellow to buff coloured powder; it has a sweet taste with a slight background of bitterness. In the pure form it is not very hygroscopic but if impurity is present, the substance is very hygroscopic, the resulting wet mass becoming organge-brown on continued exposure to air. It is very soluble in water, methanol and ethanol but is almost insoluble in acetone, chloroform, carbon tetrachloride and dry ether.

Melting Point and Ultra-violet Light Curve

The melting point was taken on a Kofler block and the behaviour of the substance observed microscopically under crossed nicols. At 110–120° the amorphous powder swelled and at about 150° it became brightly crystalline; these crystals melted at 154–157°. The ultra-violet light curve showed peaks at 266–268, 296 and 325 mμ.

Effect of Hydrolysing Conditions

The compound is stable in warm water (60–70°) for periods up to 10 hours. When heated in a boiling water bath with hydrochloric acid a very small quantity of free anthraquinones is produced. We have used strengths ranging from 0·1N to 3·5N and time of heating 15–30 minutes and though there is evidence that the yield of anthraquinone increases with increasing time of boiling, the yields are always low. But, there is evidence that the molecule has been effected, even by mild acid conditions, and this fact will be discussed in a subsequent paper.

Oxidation with Ferric Chloride

A few mg. of the compound were heated with 15 ml. of 3N HCl containing 25 per cent $FeCl_3$ in a boiling water bath for 15 minutes. The solution was cooled, extracted with carbon tetrachloride and the latter examined for the presence of anthraquinones by the following three methods. (*a*) On shaking a portion with NaOH solution a bright rose pink colour resulted, indicating the presence of anthraquinones: the amount present was a great deal more than that obtained when a similar quantity of Compound A was hydrolysed with acid alone as already described. (*b*) Another portion of the carbon tetrachloride extract was chromatographed by the method already described[2]: two spots corresponding to aloe-emodin and to chrysophanol were obtained. The spot corresponding to chrysophanol ($R_F = 0{\cdot}93$) was eluted and paper chromatographed, along with authentic chrysophanol, using the system devised by Hillis[7]. An R_F value of 0·77 was obtained (authentic chrysophanol 0·76). (*c*) The bulk of the carbon tetrachloride solution was concentrated and allowed to cool; crystals appeared and were shown to be aloe-emodin by position on the chromatogram[2], melting point 223°, and ultra-violet light spectrum which was identical with that of authentic aloe-emodin. The second anthraquinone present was much more soluble in carbon tetrachloride and it was difficult to get suitable crystals; the ultra-violet light spectrum of those obtained indicated that impurities were present. The melting point was 184° which is consistent with that of chrysophanol (m.p. 197°) containing traces of impurity.

We therefore conclude that ferric chloride treatment produces aloe-emodin and a second anthraquinone compound which may be chrysophanol.

Discussion

We have already pointed out[1] the similarities to and differences from *aloin* exhibited by Compound A and further work on this subject is being done. The relationship to *casanthranol*, isolated from cascara bark by Lee and Berger[6], has also been referred to[1]. Lee and Berger claim it is a glycoside of aloe-emodin anthranol and glucose to which is attached a methyltetrahydroxypentonic acid lactone and an hexitol group. Its molecular weight, empirical formula and optical rotation are given but it has no definite melting point. The stated properties differ from those of Compound A because the latter is stable in water, is not a true glycoside and contains chrysophanol (or a similar anthraquinone) as well as aloe-emodin. We have prepared several batches of casanthranol from cascara bark by following the published instructions, and have also prepared some from commercial casanthranol by re-crystallisation from *iso*-propanol. The physical properties of these batches and even the analysis for elements, and optical rotation corresponded to those published by Lee and Berger but in all instances they were shown to be a mixture of Compound A with a little Compound C and several other impurities. It is possible that the differences from Compound A are partly explained by the presence of the glycoside, Compound C, as an impurity in

casanthranol. We conclude that casanthranol is not a pure substance but is a purified fraction containing a large proportion of Compound A.

The presence of Compound A may also explain some anomalous results obtained in the *chemical* assay of cascara extracts. Mitchell[8] and Fairbairn[9] both claim they can obtain much higher results for the amount of combined anthraquinones in cascara extract than can be obtained by the method of Fairbairn and Mahran[10]. These higher results were obtained by the use of mild oxidising conditions (Mitchell, private communication) and it now seems obvious that the increased yields of anthraquinones is due to the oxidative breakdown of Compound A.

Finally the question of the relationship between Compound A and the *pharmacological activity* of cascara is important. We have not yet been able to devise a satisfactory method of chemical assay for Compound A but a preliminary experiment based on our paper chromatographic method[2] suggests that the amount present in the bark is of the order of 5 per cent. This indicates that Compound A is the major anthraquinone compound of cascara and we hope to investigate its pharmacological activity in order to establish whether the activity of the drug is mainly due to this substance.

Acknowledgements. We would like to express our sincere thanks to Westminster Laboratories Ltd. (London) for generously providing a maintenance grant to one of us (V. K. M.) and to S. B. Penick and Co. (New York) for a supply of commercial casanthranol.

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DISCUSSION

The paper was presented by DR. J. W. FAIRBAIRN.

THE CHAIRMAN. No elementary analyses were given for compound A, and the fact that it gave only a single spot on a paper chromatogram was submitted as evidence of chemical purity. Had the chromatographic work been done in more than one solvent system? It was stated that the compound was not a glycoside. Had the mixture obtained by hydrolysis with hydrochloric acid and ferric chloride been examined for the presence of sugars?

DR. W. MITCHELL (London). Lee and Berger had described casanthranol as a complex glycoside. He had found that casanthranol was not split by simple acid hydrolysis. He did not believe that ferric chloride

acted as an oxidising agent but thought that the reaction was fairly specific to iron, whether in the ferrous or the ferric state. Other oxidising agents seemed to have little or no effect, nor did nickel and cobalt.

MR. J. H. OAKLEY (London). Had the author any information on the stability of compound A in mild alkaline conditions? He had in mind the use of magnesium oxide—in preparing elixir of cascara. Was there any difference in the yield of compound A from old and new bark?

DR. T. E. WALLIS (London). It was stated that the amorphous powder swelled and became brightly crystalline. What did this mean? Did the whole become crystalline or were crystals embedded in the amorphous matrix? Did the crystals separate?

MR. C. A. JOHNSON (Nottingham). Was any information available on the pharmacological activity of the new compound and was it likely to be the main contribution towards the total activity of cascara?

MR. J. H. OAKLEY (London). Did the information about the melting point give a clue to a method for obtaining crystalline material?

DR. FAIRBAIRN replied. He hoped to co-operate with an organic chemist in elucidating the structure of compound A. They did not claim that the material was 100 per cent pure, and they realised that one spot on a chromatogram was not a good criterion of purity. They had used two systems; that described, and one using *iso*butanol-acetic acid in water, and and in both there was only one spot. As compound A was not easily split in acid conditions, he assumed it was not a true glycoside. He had tested the hydrolysate and found sugars present. They had not yet studied the stability of the compound in detail, but they had so far avoided alkaline conditions. They had used old bark. Loss of the griping effect was asserted to take place after 5 months, and no bark arrived in this country until after that time. They had used a sample of fresh bark grown here to compare the constituents with those in the commercial drug. Compound A had been observed under the microscope under crossed nicols. On warming, swelling took place and bubbles of gas were seen. Well-defined crystals then began to form which were readily visible; they melted at 155°. The activity of cascara was due to two types of glycoside—labile glycosides, which were measured by the older method, and the new compound A. His earlier method measured the labile glycosides, and corresponded well with the biological assay on mice, but appeared to represent only one-fifth of the total chemical content. Aloin had little effect on mice, and it might be that compound A also had very little effect. A quantity of compound A had been heated in a vacuum oven, and above 100° the whole mass swelled and began to decrepitate, which seemed to indicate decomposition. It did not seem a safe way of crystallising to heat to that temperature.