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The estimation of anthracene derivatives in senna and rhubarb

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An assay is described for the estimation of total anthracene derivatives in senna, rhubarb or their preparations. The extracted glycosides are oxidised by ferric chloride in aqueous solution followed by hydrolysis in acid solution. The free anthraquinones are estimated colorimetrically.

A COMPARATIVE study on the estimation of anthracene derivatives in drugs has been made recently by Müller, Christ & Kühn (1962); seven methods were examined and, from these, a new method was recommended for the colorimetric estimation of anthraquinones formed after oxidation of the anthrones and dianthrones in alkaline medium.

All the existing methods use the same general principle of extraction and hydrolysis of glycosides, followed by extraction and purification of the resulting aglycones. The reduced aglycones are oxidised to anthraquinones in alkaline medium, in which they are then estimated colorimetrically. The extraction, hydrolysis and purification stages in this process are well established, but variations still result from the oxidation stage, the details of which must be precisely followed in order to obtain reproducible results. This oxidation is now studied and a method is recommended based on a new principle of oxidation.

OXIDATION OF SENNOSIDES BY FERRIC CHLORIDE

The starting point for the investigation was the ferric chloride oxidation of sennosides or sennidins A and B to rhein as published by Stoll, Becker & Kussmaul (1949), and recently applied by Fairbairn & Simic (1963, 1964) to the estimation of C-glycosides in aloes and cascara.

This method of oxidation of sennoside is attractive because, in theory, only rhein can be formed; whereas the oxidation of sennidins with oxygen or hydrogen peroxide in alkaline medium results in a mixture of products. This may be demonstrated by paper chromatography and ultraviolet spectrophotometry as used by Auterhoff & Sachdev (1962) who treated emodin dianthrone with N sodium hydroxide and found that emodin, hypericin (naphthodianthrone) and other substances of unknown constitution were formed.

Using this method, a chromatogram of the aglycones of senna, obtained after oxidation in alkaline solution with hydrogen peroxide, showed different spots with variable colours, and after acidifying this solution and extracting with ether, only 60% of the theoretical amount of aglycones was obtained. The ether insoluble part was an amorphous brown powder, probably composed of polymerised anthrones and hypericin-like compounds. The amount of these different substances formed during

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oxidation will depend on temperature, oxygen, light and concentration, thus explaining the difficulty of obtaining reproducible values for $E(1\%, 1\text{ cm})$ of the reaction product and the variability of the maxima of absorption reported by different authors.

If the sennidin could be split into two molecules of rhein then a constant $E(1\%, 1\text{ cm})$ value would be obtained. In an attempt to achieve this, ferric chloride and other metal salts (Hg, Cu, Cr, Ce) were tested in different concentrations (5–25%) in strong acid medium (>2 N). The sennosides were never completely split and the product contained rhein and sennidin together with other substances which gave a positive Bornträger reaction but with a much lower $E(1\%, 1\text{ cm})$ value. On a chromatogram these substances were located below the spot of rhein.

The incomplete conversion of sennoside to rhein may be explained by the fact that in a strong acid medium it is hydrolysed before it is oxidised, so forming the insoluble sennidin, which is not attacked by ferric chloride. The other substances formed are intermediates between sennidin and rhein, they are insoluble and remain at their respective oxidation steps, e.g. dehydrodianthrone (Hörhammer, Wagner & Kohler, 1959). Brockmann & Eggers (1955) have also shown that the oxidation of a dianthrone in the presence of light gives rise to the formation of a series of intermediates. It was therefore concluded that the oxidation must be effected before the hydrolysis of the glycosides in order to obtain a complete oxidation of sennosides into two molecules of rhein glucoside. The sugar moiety of the molecule also probably exerts a protective action on the anthraquinone structure.

The conversion of sennosides with ferric chloride in weak acid medium (<0·5 N) was tried. Although the results were better, the conversion was not complete. The complete oxidation of the sennoside to rhein glucoside appeared to be achieved quickly by the use of ferric chloride without addition of any acid. After detailed examination the concentration of $FeCl_3.6H_2O$ was found to be optimal between 5 and 10%. Finally a reaction mixture containing 6·6% of $FeCl_3.6H_2O$ was adopted because with higher concentrations it was difficult to redissolve the precipitated ferric hydroxide at the later stage in the process.

Fig. 1 gives the oxidation and hydrolysis curves of sennoside A by the action of 6·6% $FeCl_3.6H_2O$ in water at 100°. The rate of hydrolysis of sennoside to sennidin is slow and oxidation to rhein glucoside takes place before the hydrolysis. After 20 min of oxidation, 33·1% of the sennoside was split and hydrolysed into rhein, the remaining 66·9% being rhein glucoside, as shown by paper chromatography. The complete hydrolysis of this rhein glucoside was achieved in 0·4 N hydrochloric acid by further warming for 20 min at 100°. The end product was pure rhein as shown by the E (1%, 1 cm) value, paper chromatography and ultraviolet absorption curve.

The conversion of sennoside A to rhein by this method was found to be 95·0%. For pure, dry rhein E (1%, 1 cm) in N potassium hydroxide is 335. The conversion of sennosides to rhein gives a theoretical figure of 221 whilst with the proposed conversion procedure for dry sennoside A

a value of 210 was obtained. A 100% conversion cannot be expected because the sennosides are not pure substances. Thin-layer chromatography showed the presence of at least 3 spots in sennoside A and 5 spots in sennoside B; sennoside A was therefore used in this investigation.

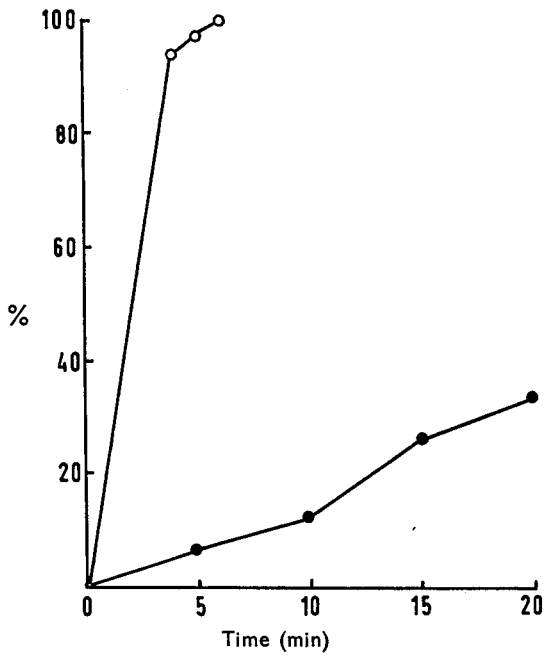

FIG. 1. Hydrolysis (●) and oxidation (○) (%) of sennoside A in water at 100° containing 6·6% $FeCl_3$ 6.H_2O.

Hence with the usual methods of oxidation the λ_{max} is variable and the E (1%, 1 cm) varies from 130 to 170. With ferric chloride in strong acid the λ_{max} is also variable with an E (1%, 1 cm) of 160–180, but using ferric chloride as described the λ_{max} is precisely 500 mμ with an E (1%, 1 cm) of 221.

APPLICATION TO SENNA AND RHUBARB

For the application of the ferric chloride oxidation method to the estimation of total anthracene derivatives in senna and rhubarb, it was necessary to test the different phases of the procedure.

Complete extraction of the glycosides from the powdered drugs can be obtained in 15 min by boiling water (Dequeker, 1962) if the drug: water ratio is no more than 1:200. The amount of glycosides in the oxidation step must never be more than 2 or 3 mg for each 20 ml of the 10% ferric chloride solution and exposure to light should be avoided. A reference standard was made using 5 ml (=1 mg sennoside A) of a solution of 20 mg sennoside A in 100 ml water, adding a trace of sodium bicarbonate.

During oxidation a precipitate of ferric hydroxide is formed and this must be completely redissolved by frequent shaking in the subsequent hydrolysis stage in 0·4 N hydrochloric acid over a period of 20 min. In the hydrolysis, the use of strong acids should be avoided because they react with extractive matter to produce a large quantity of amorphous material which renders the extraction of aglycones difficult. In the proposed method, no brown material is formed and the extraction of aglycones is simple, even with preparations such as syrups containing other plant extracts.

In the method the total anthracene derivatives are determined. The free forms can be removed before oxidation and hydrolysis by extraction with chloroform.

An estimation of the glycosides soluble in sodium bicarbonate has been omitted. Such an estimation has little value since I have shown the presence of the heterodianthrones sennoside C and rheidin A in the leaves and the pods of senna and the complete series of heterodianthrones in rhubarb (Lemli, 1963, 1964); several of them are only slightly soluble in sodium bicarbonate.

The percentage of glycosides is expressed as sennoside A. It may also be expressed as rhein because rhein accounts for about 80% of the total anthraquinones formed (Lemli, 1963).

For convenience, the results of the rhubarb estimation are also expressed as rhein, although different anthraquinones are present, and it has been assumed that the molecular absorptions of the different anthraquinones are approximately the same.

The chromatograms of the anthraquinones obtained when this method was applied to senna leaves and to rhubarb show only the spots of the different oxidised anthraquinone derivatives.

PROCEDURE FOR THE ASSAY OF TOTAL ANTHRACENE DERIVATIVES IN SENNA PODS AND LEAVES, RHUBARB, AND THEIR PREPARATIONS

Reagents. 10% ferric chloride solution: a solution of 10% $FeCl_3.6H_2O$ A.R. in water (w/v). Hydrochloric acid containing not less than 37% HCl. Ether. N Potassium hydroxide.

Procedure. Weigh 100 mg of the drug in moderately fine (No. 44) powder, in a 100 ml conical flask with ground joint and mix with 20 ml water. Weigh and attach a condenser and warm for 15 min in a boiling water-bath (water level must be above the liquid level in the flask). Cool, and weigh and adjust to the original weight with water. Centrifuge. Transfer 10 ml of the clear solution to a 100 ml round bottom flask with ground joint and add 20 ml of 10% aqueous solution of ferric chloride with mixing. Attach a condenser, immerse the flask to the neck in a boiling water-bath and avoiding direct illumination of the flask, warm for 20 min. Add 1 ml hydrochloric acid, mix thoroughly and continue warming for another 20 min, shaking frequently to dissolve the precipitated ferric hydroxide completely. After cooling, transfer quantitatively to a separating funnel and extract with three portions each of 25 ml ether,

rinsing the flask with each portion of ether. Wash the combined ether layers with 25 ml water. Transfer the ether to a 100 ml volumetric flask and make up to volume with ether. Evaporate 10 ml of this ether solution in a conical flask and dissolve the residue in 10 ml N potassium hydroxide, avoiding direct light. Measure the absorption of this solution at 500 mμ in a 1 cm cell, against N potassium hydroxide. Using the value E (1%, 1 cm) sennoside A = 221, or E (1%, 1 cm) rhein = 335 calculate, as equivalent of sennoside A or rhein, the percentage of total anthracene derivatives present in senna pods or leaves or their preparations. For rhubarb and preparations, calculate the percentage of total anthracene derivatives present as an equivalent of rhein.

When examining a tincture, extract or other fluid galenical preparation, the extraction step is omitted and the assay begun by mixing the preparation directly with water and ferric chloride solution.

Experimental

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The method for the separation of the anthraquinones was that described by Kinget (1963). Spots were made visible with 5% potassium hydroxide in 50% ethanol with warming at 100°.

OXIDATION AND HYDROLYSIS OF SENNOSIDE A BY FERRIC CHLORIDE

The oxidation curve was obtained as follows. To 20 ml of a solution containing 4 mg sennoside A in water was added 14 ml water and 66 ml of a 10% solution of $FeCl_3.6H_2O$. The mixture was warmed in a boiling water-bath and after 4, 5 and 6 min a sample of 20 ml was removed. To each sample was added 4 g Na_2HPO_4 (in 40 ml water) and the precipitate filtered off. The filtrate was then acidified and extracted with ether, which now contained only rhein as shown by paper chromatography. To the acidified filtrate, containing the remaining glycosides, was added sufficient sulphuric acid to obtain a 5 N solution and the glycosides were hydrolysed by heating in a boiling water-bath for 15 min. The resulting aglycones were extracted and the quantity of unoxidised sennidin determined by densitometry (Lemli, 1963). The sennidin concentrations after 4, 5 and 6 min were respectively 5·4, 3·2 and 0%.

The hydrolysis curve was established in the following manner. A mixture as described above was warmed in a boiling water-bath and 20 ml samples were taken after 5, 10, 15 and 20 min. The samples were cooled and extracted with ether to remove the liberated aglycones. Paper chromatography showed that the aglycone formed was rhein. After extraction with ether the solutions were further warmed for 20 min for complete oxidation and for another 20 min after addition of concentrated hydrochloric acid for complete hydrolysis. The aglycones were extracted and determined by colorimetry. The percentages of unhydrolysed sennoside after 5, 10, 15 and 20 min were respectively 93·1, 88·2, 73·5 and 66·9%. These results are represented graphically in Fig. 1.

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