VEGETABLE PURGATIVES CONTAINING ANTHRACENE DERIVATIVES

PART VIII.—THE PAPER CHROMATOGRAPHY OF CERTAIN ANTHRAQUINONES AND THEIR GLYCOSIDES

BY T. J. BETTS, J. W. FAIRBAIRN AND V. K. MITAL

From the Pharmacognosy Research Laboratory, School of Pharmacy, University of London

Received February 28, 1958

A simple paper chromatographic technique, using toluene alone as a running solvent, is described for identifying free anthraquinone compounds in crude drugs. A second system is described for anthraquinone glycosides: the running solvent is the lower aqueous phase of a water:acetone:benzene mixture. This system was found particularly successful for cascara bark *Rhamnus purshiana* D.C. as it readily revealed the presence of at least three anthraquinone glycosides.

METHODS of estimating the amount of glycosidal and free anthraquinones in several drugs have been published in this series[1–3]. They were designed to evaluate crude drugs and their extracts but did not enable individual glycosides to be estimated separately. As we wish to obtain more information on the distribution and quantities of individual anthraquinone compounds in these drugs we have investigated the possible application of paper chromatography. As a result suitable methods of separating and identifying some of the individual substances have been developed.

About seven papers have been published on the paper chromatography of the anthraquinones, mainly by the Japanese workers Shibata, Tsukida and Takido[4–8]. In all instances the glycosides are first hydrolysed with acid and then the liberated aglycones (free "anthraquinones) are examined by paper chromatography. The published methods therefore deal in practice with the paper chromatography of the free anthraquinones only. We decided not only to investigate these methods but also to attempt to devise suitable methods for separating the individual glycosides without previous hydrolysis. By this means they could be eluted from the paper in virtually the same form in which they occur in the living plant.

EXPERIMENTAL METHODS AND RESULTS

FREE ANTHRAQUINONES

Samples of aloe-emodin, aloe-emodin anthranol, chrysophanol, emodin, and rhein were prepared, authenticated and used to test the published methods. Fair agreement was found between our R_F values and those of the Japanese workers although the latter used "Toyo" papers while we used "Whatman" papers and sometimes our solvents were slightly different. But a number of irregular results were obtained and the methods were found to suffer from some defects. (*a*) A confusing number of light petroleums of boiling ranges from 45° to 110° is quoted. Each

fraction results in a different R_F value for a given anthraquinone compound. (*b*) Some of the solvent systems are very volatile and quick-running and therefore susceptible to slight variations in temperature. (*c*) It was almost impossible to avoid entrainment of one phase in another when preparing certain solvent systems; this entrainment also caused results to vary. (*d*) The spots frequently showed bad tailing.

Method Recommended for Free Anthraquinones

We found the most satisfactory of the solvent systems previously used to be toluene saturated with water. Further work on this system showed that toluene alone was equally effective; at first we assumed that the paper contained enough moisture to saturate the toluene during use, but when paper which had been dried in an oven for a few hours was used equally satisfactory results were obtained. Results can be obtained

TABLE I

R_F VALUES OF PURE ANTHRAQUINONES, USING TOLUENE AS THE MOBILE PHASE

Anthraquinone compound	R_F values*			
	Whatman paper No. 1	Whatman paper No. 20	Whatman paper 3 MM.	Japanese workers Toyo No. 131
Chrysophanol	0·98 (0·99)	0·91 (0·96)	0·98 (0·99)	(0·99)
Aloe-emodin	0·65 (0·80)	0·58 (0·65)	0·65 (0·80)	(0·87)
Aloe-emodin anthranol ..	0·66 (0·84)	0·56 (0·65)	0·67 (0·88)	—
Emodin	0·40 (0·50)	0·32 (0·40)	0·40 (0·50)	(0·63)
Rhein	0	0	0	(0·03)

* R_F values in brackets were measured from the front of the spot.

after one hour with a simple ascending technique. The paper after removal from the tank and drying is sprayed with a 0·5 per cent solution of magnesium acetate in methanol and heated at about 90° for a few minutes; anthraquinone compounds become pink to red in daylight and ultra-violet light. Table I shows the R_F values obtained with pure anthraquinones using toluene as the mobile phase, three varieties of Whatman paper and a range of temperatures between 18° and 22°. Whatman No. 20 paper gives a better separation as it slows down this quick-running solvent more than the other papers.

"Tailing" of the spots still occurred which makes this method unsuitable for quantitative work but if care is taken not to overload the paper the "tailing" can be sufficiently reduced to measure the R_F values in the usual manner. Where "tailing" was pronounced the R_F value was measured from the front of the spot as was done by the Japanese workers. In Table I we record both R_F values for each anthraquinone. The figures quoted are the average of several determinations which showed some variation, but the relative position of the spots was always the same. The ratio of the R_F values was also fairly constant, for example that for aloe-emodin and emodin was about 1·7 to 1. The R_F values for chrysophanol and rhein are inconvenient, values greater than 0·9 or less

than 0·1 being unreliable; fortunately rhein has a more suitable value in the next solvent system we discuss and Hillis[9] quotes an R_F value of 0·71 for chrysophanol. He used Whatman 3 MM paper at 22° and his running solvent was methanol saturated with *n*-heptane at 10°. We have confirmed this observation but found that aloe-emodin and emodin have similar R_F values to each other and to chrysophanol. However, the system can be used to confirm the presence of chrysophanol by eluting the spot from the toluene chromatogram and running it in this second system.

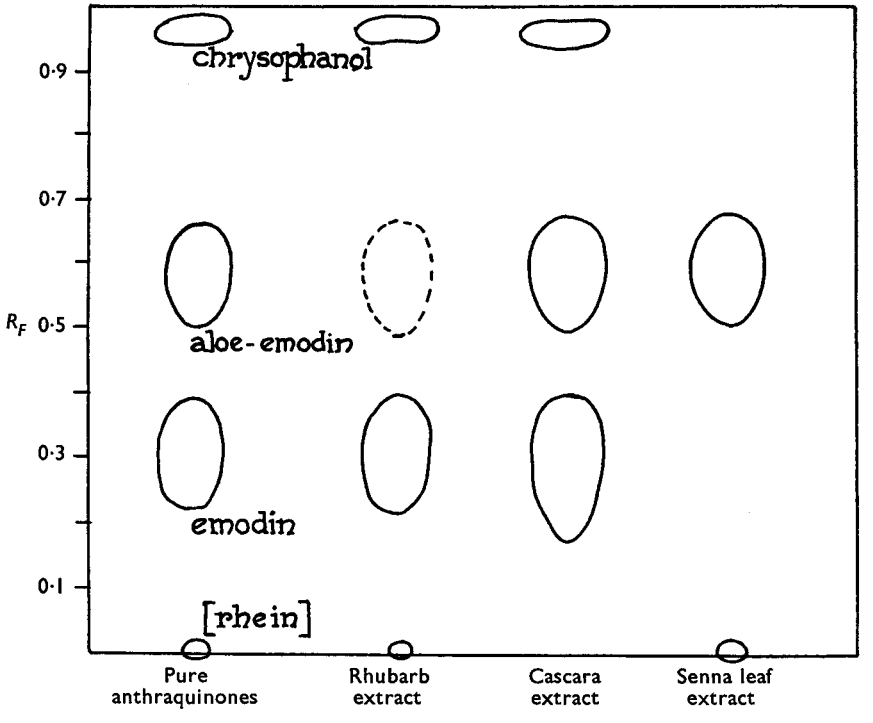

FIG. 1. Paper chromatogram of free anthraquinones and of suitable extracts of certain crude drugs. Running solvent, toluene. Whatman No. 20 paper. Temperature about 20°.

Application to Crude Drugs

The modified Bornträger test[10] was applied to powdered samples of cascara bark, rhubarb and senna leaf and the resulting benzene solutions were treated by the method described above. The results are shown in Figure 1 and indicate the free compounds present in these crude drugs.

COMBINED ANTHRAQUINONES

Danilovic[11] has published a paper chromatographic method for dealing with the glycosides as such, and he claims to be able to separate and identify the glycoside frangulin. He used the system *iso*amyl alcohol : *iso*-butyric acid : acetic acid : water. This system was found by us to be

unsatisfactory as unless the proportions were carefully controlled the four liquids formed one phase; even when two phases formed there was marked entrainment of one phase in another. The resulting chromatograms showed tailing of the spots and when the method was applied to cascara extracts no glycosides were visible on the chromatograms. Krogerous and others[12] also have published an account of the chromatography of frangula glycosides using a single phase solvent mixture of butanol: acetic acid: water. This system achieved a separation of glycosides from cascara extracts but did not give such good separation as the one we describe below. Furthermore, some of the glycosides are decomposed by the acid solvent system.

Method Recommended for Combined Anthraquinones

Our observation that rhein frequently had an R_F value similar to some of our glycosidal material led to a re-investigation of Takido's water: acetone: benzene system[6], which had proved satisfactory for rhein, and we found that, after suitable modification, it was successful for the combined anthraquinones present in cascara bark, aloes and to a lesser extent for senna pod, senna leaf and pure sennosides. The R_F values for some free anthraquinones in this system were also determined. The details are as follows. Water, acetone and benzene (2:1:4) are shaken vigorously and allowed to separate. The lower aqueous phase (the running solvent) is placed on the bottom of a chromatographic tank and a beaker containing the upper phase is also placed in the tank, which is then sealed, and allowed to come to equilibrium overnight. Meanwhile a small quantity of the substance to be investigated is transferred to the starting line of a chromatographic paper which is then placed in the tank, preferably as a cylinder, with its lower end resting in the aqueous phase. The paper is allowed to remain in the re-sealed tank for 2–3 hours or until the solvent front has reached a suitable height (ascending technique). The paper is removed, dried and sprayed with 0·5 per cent magnesium acetate in methanol and heated at about 90° for a few minutes. Combined anthraquinones became yellow to orange when sprayed, and appear in colours ranging from yellow to red in ultra-violet light. Whatman No. 1 and 3 MM papers were suitable but with Whatman No. 20 paper the solvent ran slowly. The R_F values quoted are therefore those obtained using Whatman No. 1 or 3 MM paper.

Application to Cascara Bark

Applied to cascara bark (*Rhamnus purshiana* DC.) the method revealed the presence of at least three anthraquinone glycosides or similar compounds.

A quantity of powdered bark was exhausted with chloroform to remove free anthraquinones, chlorophyll, etc., and dried. When the dried marc was extracted with water and this solution chromatographed by the method described two glycosidal spots were observed and were named Compound A ($R_F = 0{\cdot}87$) and Compound B ($R_F = 0{\cdot}74$). When a methanolic extract was treated in a similar manner spots corresponding to

Compound A and a third glycoside, Compound C ($R_F = 0{\cdot}45$) were revealed. These three substances were shown to contain anthraquinone compounds by preparing adequate quantities of each, using band chromatography and cutting out the appropriate bands, which were then eluted with methanol. A portion of each methanolic solution was heated in 3N HCl at 98° for 15 minutes and a second portion was heated in 3N HCl, containing 25 per cent of ferric chloride at 98° for 15 minutes. Each aqueous portion was extracted with carbon tetrachloride and the latter solutions treated by the method of paper chromatography for the free anthraquinones. Both Compounds B and C produced free anthraquinones by direct hydrolysis in acid, but Compound A produced free anthraquinones only after ferric chloride oxidation[13].

TABLE II

APPLICATION OF THE PROPOSED PAPER CHROMATOGRAPHIC METHOD TO ANTHRAQUINONE GLYCOSIDES

Compound	R_F value
Sennoside A, "free"	0·96
Sennoside A, Na salt	0·86
Sennoside B, "free"	0·96
Sennoside B, Na salt	0·90
Rhein	0·75
Rhein, Na salt ..	0·69
Aloin (m.pt. 142–5°)	0·66
Emodin	0·26*
Aloe-emodin ..	0·18*
Chrysophanol ..	0·0

* Values measured from front of spot.

Application to other Anthraquinone Glycosides

The method was applied to certain pure glycosides and a few free anthraquinones and the results obtained are shown in Table II.

When applied to senna pod and leaf, spots corresponding to the sennosides were observed. However as the R_F values are rather high it was concluded that the method is not as suitable for investigating the glycosides of senna as it is for those of cascara.

DISCUSSION

This work was undertaken in order to obtain a more detailed picture of the anthraquinone compounds present in certain drugs, than could be obtained by our earlier methods of investigation. The two methods have enabled this to be done on a qualitative basis and we have found them particularly useful in the investigation of cascara bark constituents. The methods are simple and results can be obtained fairly quickly; this is particularly true of the method for free anthraquinone using toluene alone as the running solvent. Another important advantage is the fact that the methods require only small quantities of material which makes it possible to use small samples from the living plant for biochemical investigations. No attempt has been made to make the method quantitative but we believe that, by carefully controlling the conditions, the

methods described could be used for quantitative estimations. An example of this is the recent work of Paris and Durand[14], who have devised a quantitative paper chromatographic method for aloin; they controlled the conditions carefully so that the spots were sufficiently well defined to be measured by a suitable densitometer.

The range of compounds we have examined is small but the results indicate that the R_F values are related to chemical structure. In both systems (Table I and II) the order is rhein, emodin, aloe-emodin and chrysophanol, rhein having the highest R_F value when a polar running solvent is used and chrysophanol having the highest value when a non-polar solvent is used. These properties run parallel with the reactivity of the substituent in position 3 of the molecule (β-position). All four compounds have two α-hydroxyl groups but rhein has a carboxylic group in the β-position; emodin an hydroxyl, aloe-emodin a primary alcohol and chrysophanol a methyl group. It is known that a β-hydroxyl group is more acidic than an α-hydroxyl[15] so that the order rhein, emodin, aloe-emodin, chrysophanol appears to represent an order of decreasing acidity, or reactivity of the β-hydroxyl group. This would be expected to affect the solubility in polar and non-polar solvents in the same order.

Acknowledgements. We would like to express our sincere thanks to the Westminster Laboratories Ltd. for generously providing a maintenance grant to one of us (V. K. M.).

REFERENCES

1. Fairbairn and Michaels, *J. Pharm. Pharmacol.*, 1950, **2**, 807.
2. Fairbairn and Lou, *ibid.*, 1951, **3**, 93.
3. Fairbairn and Mahran, *ibid.*, 1953, **5**, 827.
4. Shibata, Takido and Tanaka, *J. Amer. chem. Soc.*, 1950, **72**, 2789.
5. Shibata and Takido, *J. pharm. Soc. Japan*, 1952, **72**, 1311.
6. Takido, *Pharm. Bull. Japan*, 1956, **4**, 45.
7. Tsukida, Suzuki and Yokota, *J. pharm. Soc. Japan*, 1954, **74**, 224.
8. Tsukida and Suzuki, *ibid.*, 1955, **75**, 982.
9. Willis, *Aust. J. Chem.*, 1955, **8**, 290.
10. Fairbairn, *Pharm. J.*, 1942, **148**, 198.
11. Danilovic, *Acta Pharm. Jug.*, 1953, **3**, 219.
12. Krogerus, Maki and Sylvander, *Farm. Notisbl.*, 1957, **66**, 115.
13. Fairbairn and Mital, *J. Pharm. Pharmacol.*, 1957, **9**, 432.
14. Paris and Durand, *Ann. pharm. franç.*, 1956, **14**, 755.
15. Thompson, *The Naturally Occurring Quinines*, London, 1957, p. 159.