STUDIES IN THE CHROMATOGRAPHY OF SENNA AND RELATED COMPOUNDS

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The chromatographic examination of vegetable drugs is only now beginning to receive detailed study, and so there are few references to this method as applied to senna or other anthraquinone-containing drugs. Ernst and Weiner[1] used a column of magnesia, and stated that, because of its alkalinity, it had the advantage of causing the emodin-containing layer to be coloured red. Gibson and Schwarting[2] in a paper on the chromatographic isolation of the trihydroxymethylanthraquinones of cascara sagrada used a mixture of 3 parts of celite and 1 part of magnesia as the adsorbing agent. They had difficulty in differentiating the anthraquinone zones, and therefore carried out comparative experiments using dilute chloroform solutions of the pure anthraquinones which they anticipated would be present. On chromatographing a chloroform solution containing the three pure substances, emodin, aloe-emodin and isoemodin, continued washing of the column with chloroform over a period of a week was required before differentiation of the initial red layer was evident. Cropper[3] recommended heavy magnesium carbonate as an adsorbent for hydroxyanthraquinone derivatives. No work appears to have been published concerning the behaviour of simple anthrones on chromatographic columns.

Experimental

Chromatography of Senna Extracts

Chloroform Extract. 10 g. of powdered Tinnevelly senna leaves was completely extracted with chloroform in a Soxhlet apparatus. The extract (about 20 ml.) was chromatographed on a column of alumina 45 cm. long and 2 cm. diameter. The result is shown in Figure 1. Development was accomplished by addition of more chloroform, the lowest layer passing completely into the filtrate. After extrusion, the different zones were treated as follows.

Zone 1—Red. After many trial experiments, continuous extraction with ether was found to be necessary to elute the adsorbed substance. The resulting yellow solution was concentrated and allowed to evaporate. A yellow oily residue (orange in ultra-violet light) remained. This residue gave a negative reaction for glycosides by Molisch's test, and for free anthraquinones by Fairbairn's modification of Bornträger's reaction[4].

Zone 2—Yellow. Easily eluted with alcohol. The solution was concentrated and allowed to evaporate spontaneously. The yellowish residue gave negative reactions for glycosides and anthraquinones.

Zone 3—Green in daylight and red in ultra-violet light. These colours are characteristic of chlorophyll, so this zone was not further examined.

Zone 4—Yellow. Negative reactions obtained for glycosides and anthraquinones.

Alcohol Extract. An alcoholic extract, prepared similarly to the chloroform extract, was chromatographed on a column of alumina 25 cm. × 2 cm. The result is shown in Figure 2. On developing with alcohol, the yellow layer filtered through completely, but the three upper zones remained strongly adsorbed. Chloroform was no more successful. On

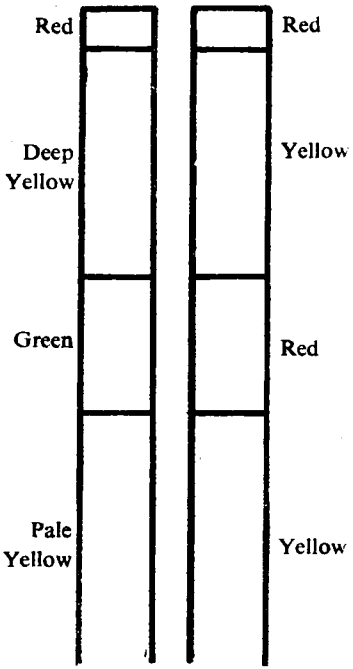


Fig. 1. Chromatograms of chloroformic extract of senna on alumina. Left, in daylight; right, in ultra violet light.

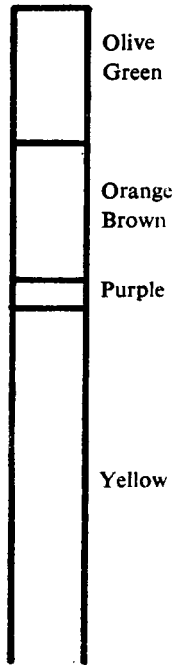


Fig. 2. Alcoholic extract of senna on alumina in daylight.

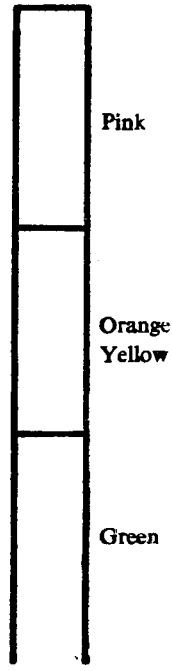


Fig. 3. Chloroformic extract on heavy magnesium carbonate and potato starch, in daylight.

adding water, a yellow zone washed through from the upper zones and the purple zone disappeared, leaving three zones—1 green, 2 orange, 3 yellow. After extrusion the zones were examined.

Zone 1—Green. Continuous extraction with hot alcohol was necessary for elution. After evaporation of the solvent an oily residue remained, which gave a positive reaction for glycosides and also for both free and combined anthraquinones.

Zone 2—Orange. Eluted similarly to Zone 1, giving a yellowish oily residue. This gave a positive reaction for glycosides but negative for anthraquinones.

Zone 3—Yellow. When similarly treated negative reactions were obtained for both glycosides and anthraquinones.

Search for less powerful adsorbents. Chromatographic columns were prepared, using magnesium carbonate, magnesium oxide, calcium hydroxide, calcium carbonate, prepared chalk, and kieselguhr, but all were in such a fine powder that it was only with the greatest difficulty that the liquid percolated. Dilution of these substances with lactose or potato starch effected some improvement, but was not entirely satisfactory.

A chloroform extract of Tinnevelly leaf, prepared as formerly, was chromatographed on alumina as before. The red zone was eluted with hot chloroform. The eluate was chromatographed on a column composed of heavy magnesium carbonate 1 part and potato starch 3 parts, and developed with methyl alcohol. Figure 3 shows the result. The bottom green zone filtered through.

Zone 1—Pink. The magnesium carbonate was dissolved in dilute sulphuric acid and the liberated adsorbate extracted by shaking with chloroform in a separator. The chloroform solution gave a positive reaction for anthraquinones.

Zone 2—Orange Yellow. Similar treatment showed the presence of anthraquinones.

Zone 3.—Green filtrate. Anthraquinones absent.

The use of neutral alumina. It was thought that the slightly acidic nature of the hydroxyanthraquinones present in senna might result in combination with free alkali present in the alumina, and might be, at least partly, the cause of the firm adsorption. Before use, therefore, the alumina was washed with water containing a little hydrochloric acid, and then with water until the washings were neutral. In order to reduce still further the adsorptive power of the alumina, the wet material was washed several times with methyl alcohol, drained well, and air dried, as described by Williams[5]. This material was used for the following chromatogram.

30 g. of powdered Tinnevelly leaf, which had previously been completely extracted with chloroform (and therefore contained no free anthraquinones) were extracted with alcohol in a Soxhlet apparatus. The resulting extract was chromatographed on a column of alkali free alumina measuring 40 cm. × 2 cm. with the result shown in Figure 4. On addition of more alcohol, partial development took place, and the lowest zone filtered through. Elution of the other zones was difficult, and all required continuous extraction with methyl alcohol. The following reactions were given by the eluates.

Test	Zone 1	2	3	4	5	6
Bornträger's	+	+	+	−	−	−
Molisch's	−	+	+	+	+	−

"Blocking" of hydroxyl groups by acetylation. Although the use of the neutral alumina gave separation into a larger number of zones than

the other adsorbents, the elution of these zones still presented considerable difficulty. Many hours' continuous extraction in a Soxhlet apparatus was necessary. In order to try and reduce this great adsorbency, it was decided to acetylate the material before chromatographing.

An alcoholic extract was prepared from 20 g. of powdered Tinnevelly leaf, and the alcohol distilled off. A soft extract weighing about 6 to 7 g.

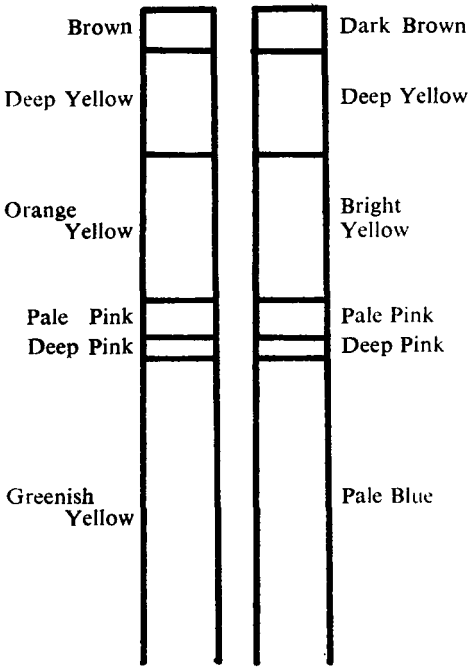


Fig. 4. Alcoholic extract of leaf exhausted with chloroform, on alumina. Left, in daylight; right, in ultra violet light.

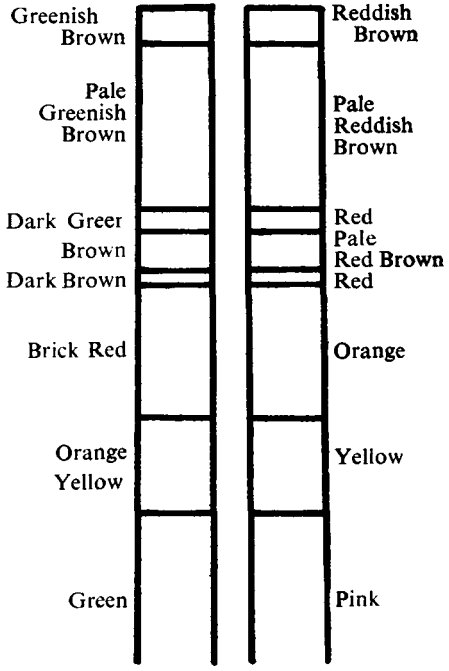


Fig. 5. Acetylated extract on neutral alumina. Left, in daylight; right, in ultra violet light.

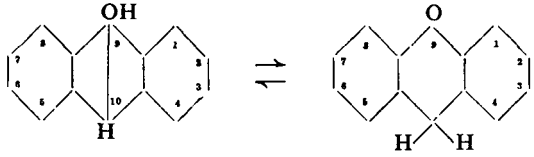
remained. To this was added 14 ml. of acetic anhydride containing 2 drops of concentrated sulphuric acid, and the mixture was heated on a water-bath for 30 minutes. It was poured into water, and the resulting precipitate filtered, washed and dried.

The acetylated extract was found to be incompletely soluble in light petroleum, ether and alcohol. Almost complete solution was obtained in acetone, so an acetone solution was prepared, filtered, and chromatographed on neutral alumina (column 40 cm. × 2 cm.), the appearance of the column being shown in Figure 5. On addition of more acetone, the lowest (green) zone filtered through. Elution was found to be very difficult, the usual solvents being unsuccessful. It was found necessary to separate the zones after extrusion and extract repeatedly with warm acetic anhydride. The solution was concentrated under reduced pressure, poured into water, and the resulting precipitate filtered off, washed with

water, and dried. Zones 1 to 4 all yielded substances which gave a positive reaction for anthraquinones.

Chromatography of Known Related Substances

Owing to the great difficulty experienced in development and elution of the adsorbed substances present in the various extracts of senna leaves tested, it was decided to carry out chromatographic experiments on known substances of a similar constitution to those present in senna leaves.



Anthranol Anthrone

1 : 8-dihydroxyanthranol. 10 ml. of a 1 per cent. solution of 1:8-dihydroxyanthranol in benzene was chromatographed on a column of neutral alumina 15 cm. × 12 mm. with the result shown in Figure 6. On development with benzene the bottom yellow layer passed into the filtrate. The main orange zone was unaffected by methyl alcohol, ethyl alcohol, chloroform or pyridine, even when hot. The use of heavy magnesium carbonate as adsorbent gave a chromatogram of similar appearance. Elution was no easier when the usual organic solvents were used.

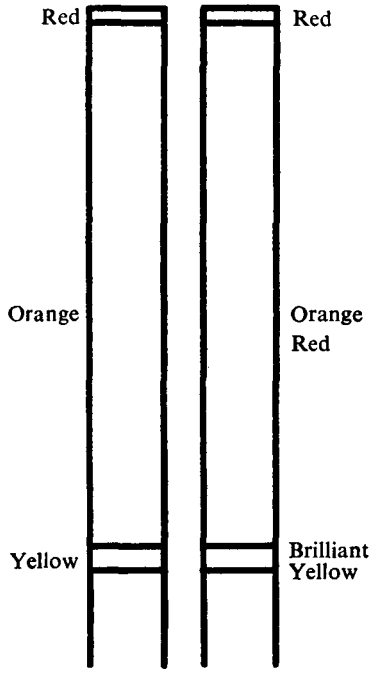


Fig. 6. 1 : 8. Dihydroxyanthranol in benzene, on neutral alumina. Left, in daylight; right, in ultra violet light.

Di-acetate of 1 : 8-dihydroxyanthranol. 1 g. of 1:8-dihydroxyanthranol was refluxed for 30 minutes with 2 ml. of acetic anhydride containing 1 drop of concentrated sulphuric acid. The mixture was poured into water, filtered and crystallised from glacial acetic acid. M.pt. 208° to 209°C. A 1 per cent. solution of the di-acetate in acetone was chromatographed on neutral alumina. The usual solvents were inefficient eluants,

but acetic anhydride was a little more active. A 1 per cent. solution in chloroform was chromatographed on heavy magnesium carbonate. There was a narrow yellowish green zone at the top of the column; the remainder of the column was yellow. On developing with chloroform the yellow zone passed into the filtrate, which had an intensely brilliant yellow fluorescence in ultra-violet light. On evaporation of the solvent, the residue had melting-point 208° to 210°C. Mixed melting-point with the original di-acetate showed no depression.

Aloin. According to Rosenthaler[6], aloin is a compound of arabinose with aloe-emodin-anthranol. It is thus very similar to the senna glycosides described by Straub and Gebhardt[7]. The aglycone may be identical, but the sugar present in the latter is stated to be dextrose. The similarity between the compounds, however, would suggest that their adsorptive properties should be very similar.

5 ml. of a 1 per cent. solution of aloin in methyl alcohol was chromatographed on neutral alumina (15 cm. × 12 mm.). The main part of the column was orange yellow, but there was a narrow greenish yellow zone at the top, and a yellow zone of the same size at the bottom. Elution was found to be very slow, but after allowing methyl alcohol to flow through the column for several hours the filtrate became yellow, and aloin was identified in it.

10 ml. of a similar solution of aloin was chromatographed on a column of magnesium carbonate of the same size. A narrow zone of pink formed at the top, the remainder of the column being yellow. On further addition of methyl alcohol, the lower zone filtered through. The filtrate, on concentration and spontaneous evaporation, gave a reddish brown residue with a melting-point above 300°C. An amyl alcohol solution of aloin was chromatographed similarly, with the same results. This suggested that decomposition of the aloin took place on the alkaline adsorbent.

Tri-acetate of Aloin. 1 g. of aloin + 2 ml. of acetate anhydride + 1 drop of concentrated sulphuric acid were boiled under a reflux condenser for 30 minutes. The product was poured into water, filtered, washed with warm water, and dried. Greenish yellow aloin tri-acetate was obtained, m.pt. 93° to 94°C. (lit. m.pt. 95° to 96°C.), yield 1·2 g.

10 ml. of a 2 per cent. solution of this in chloroform was chromatographed on neutral alumina (20 cm. × 12 mm.), with the result shown in Figure 7. On further addition of chloroform the lowest zone filtered through as a yellow liquid with a blue fluorescence in ultra-violet light. On evaporation of the filtrate a pale yellow powder was obtained, m.pt. 93° to 94°C. The mixed m.pt. with the original aloin acetate was unchanged. A similar result was obtained when the aloin acetate was chromatographed on heavy magnesium carbonate. The main yellow zone filtered through the column when eluted with chloroform, and the original substance was identified in the filtrate.

Aglycone of Aloin. 10 g. of aloin + 10 g. of borax + 100 ml. of water were boiled for 30 minutes, cooled, and acidified with hydrochloric

acid. The orange red precipitate was recrystallised from toluene and gave m.pt. 195° to 200°C. It complied with the tests given by Cahn and Simonsen[8] for the aglycone of aloin. An acetone solution was

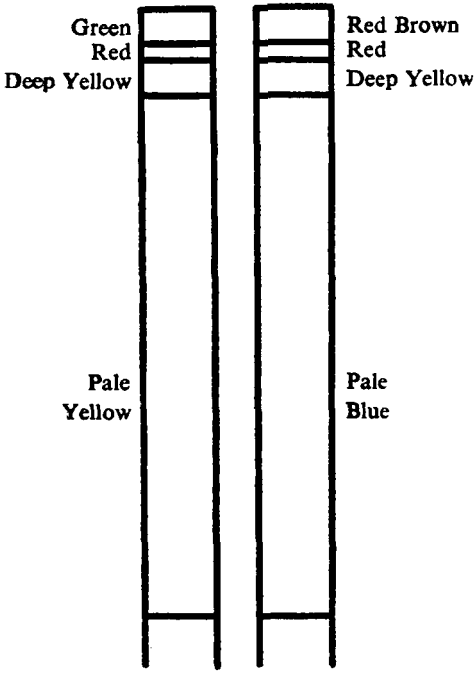


Fig. 7. Aloin triacetate in chloroform, on neutral alumina. Left, in daylight; right, in ultra violet light.

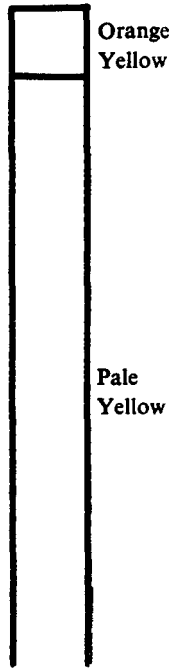


Fig. 8. 1-Chloro-9-anthrone in benzene, on neutral alumina (daylight).

chromatographed on neutral alumina. Almost the whole column was orange (red in ultra-violet light), but there was a narrow yellow band at the bottom. A satisfactory eluant was not found.

1-Chloro-9-anthrone. This substance was prepared by the method of Barnett and Matthews[9]. 1 g. of the crude product was dissolved in benzene and chromatographed on a column of neutral alumina (25 cm. × 12 mm.) with the result shown in Figure 8. No difference was observed in ultra-violet light. On addition of more benzene, the pale yellow zone filtered through. The filtrate was concentrated and allowed to evaporate. Yellow needles were obtained weighing 0·9 g., m.pt. 117°C. (lit. m.pt. 118°C.).

4-Chloro-9-anthrone. This was prepared by the method given by Barnett and Matthews[9]. 1 g. of the crude product was dissolved in benzene, and chromatographed on a column of neutral alumina as above. The whole column was yellow (orange in ultra-violet light with a narrow violet zone at the bottom). Elution was readily effected by addition of more benzene. The violet fluorescent zone was collected separately,

but on evaporation yielded little or nothing. The filtrate from the yellow zone on evaporation yielded yellow needles, m.pt. 119°C. (lit. m.pt. 118°C.), yield 0·83 g. The mixed melting-point with 1-chloro-9-anthrone was 108° to 112°C.

3-Chloro-9-anthrone. 3-chloro-anthrone dissolved completely in warm caustic soda solution, giving a yellow solution, which on addition of sodium hydrosulphite ($Na_2S_2O_4$) did not become red. It was thus free from anthraquinones. M.pt., 154° to 156°C. (lit. m.pt. 156°C.).

1 g. of the crude product was dissolved in benzene, and chromatographed on a column of neutral alumina, 40 cm. × 2 cm. The chromatogram was yellow, the upper third being slightly darker than the remainder. When viewed under ultra-violet light there was no significant change. The column was extruded and divided into three approximately equal parts, which were eluted with warm alcohol.

Bottom zone. Evaporation of the eluate gave 0·3 g. yellow residue melting between 146° and 200°C. It gave a red colour on warming with warm sodium hydroxide and sodium hydrosulphite.

Middle zone. 0·51 g. of residue, melting between 143° and 210°C. It also gave a red colour with warm sodium hydroxide solution and sodium hydrosulphite.

Top zone. 0·15 g. residue, which again gave the red colour as above, and melted between 150° and 195°C.

The three residues which had been recovered from the column were mixed, washed twice with boiling alcohol, and the insoluble residue filtered off and dried. This product was insoluble in warm caustic soda solution; on addition of sodium hydrosulphite to this, a red colour was obtained.

M.pt., 195° to 212°C.; mixed m.pt. with 2-chloroanthraquinone, 195° to 209°C. Found: C, 70·94; H, 3·48; $C_{14}H_7O_2Cl$ requires C, 69·28; H, 2·91 per cent.

The above evidence therefore points to the anthrone having been oxidised on the column of alumina, with formation of the anthraquinone.

Oxidation of Anthrone in presence of alkali. Anthrone ($C_{14}H_{10}O$) m.pt. 153° to 156°C., which was free from anthraquinone, was dissolved in warm dilute caustic soda solution, and allowed to stand in an open beaker for 3 days. A small proportion of the solution was then warmed with sodium hydrosulphite—the solution became red. The remainder of the solution was acidified with hydrochloric acid, filtered, and the precipitate washed and dried. The crude product melted between 190° and 250°C. It was washed with boiling alcohol, and the residue dried. M.pt., 235° to 247°C.; mixed m.pt. with anthraquinone, 240° to 255°C. On warming with caustic soda solution (in which it was insoluble) and adding sodium hydrosulphite, a red colour was produced.

Discussion

Alumina was first used as the adsorbent in the chromatographic examination of senna extracts. Separation into coloured zones took

place readily but elution of the adsorbed substances was very difficult. Continuous extraction with an organic solvent such as chloroform or ether was necessary to elute the zones at the top of the column which contained the hydroxyanthraquinones. The difficulty experienced in the elution of the hydroxyanthraquinones is probably due to their combination with the alumina, forming lakes (as suggested by Cropper[3]). It was found that a column prepared from a chloroform extract gave reactions for free anthraquinones only, while one prepared from an alcoholic extract gave reactions for both free and combined anthraquinones. This confirms that the glycosidal substances are insoluble in chloroform. In the examination of alcoholic extracts some of the lower zones gave a positive reaction with Molisch's Test, but gave no reaction for anthraquinones. This may have been due to the hydrolysis of some of the glycosides, and the free sugars, being less strongly adsorbed than the anthraquinones, were carried lower down the column.

The search for a more suitable adsorbent was not entirely successful. The fineness of powder was a disadvantage in many cases, resulting in extremely slow percolation of the liquid. Heavy magnesium carbonate gave most promise, one advantage being that elution can be effected by dissolving the magnesium carbonate in dilute mineral acid and extracting with chloroform in a separating funnel. One column was prepared using a mixture of magnesium carbonate 1 part and potato starch 3 parts. Through this was passed the chloroform eluate from the red (top) zone of an alumina column prepared from a chloroform extract. Development with methyl alcohol produced separation into three zones, the two top ones giving reactions for hydroxyanthraquinones, thus showing that at least partial separation of these constituents had been effected. The use of neutral alumina did allow of separation into a larger number of zones than previously, but the same difficulty was experienced in the elution of these zones.

Acetylation of the hydroxy-groups in the anthraquinones was then carried out, in order to prevent them reacting with the alumina. Although separation into six zones was achieved, elution was again very difficult.

The chromatography of single compounds, similar in nature to the active constituents of senna, was now considered. Aloin, which is an anthranol glycoside very similar in nature to those found in senna, was successfully chromatographed on neutral alumina, but on a very small scale (5 ml. of a 1 per cent. solution in methyl alcohol). Elution was very slow, and several days would have been required for elution of quantities similar to those of the senna extracts. The use of magnesium carbonate caused decomposition of the aloin, probably due to hydrolysis on the alkaline adsorbent, and possibly subsequent oxidation. Aloin triacetate was chromatographed successfully on both neutral alumina and on magnesium carbonate, thus proving that the " blocking " of the hydroxyl groups did prevent their reaction with the adsorbent.

1 : 8-Dihydroxyanthranol presented the same difficulty of elution from columns of neutral alumina and magnesium carbonate. Acetylation of this substance reduced the firmness with which it was adsorbed on

magnesium carbonate and thus rendered it suitable for chromatographing on that substance.

Three chloroanthrones were chromatographed on neutral alumina. 1-chloro-9-anthrone, and 4-chloro-9-anthrone were both successfully purified by chromatographing on neutral alumina, using benzene as the solvent and the eluant. 3-Chloro-9-anthrone when similarly treated, was partially oxidised to 2-chloroanthraquinone. Anthrone ($C_{14}H_{10}O$) was allowed to stand in dilute caustic soda solution for three days. At the end of that time it had been partially oxidised to anthraquinone.

These results, together with other evidence to be published elsewhere, suggest that certain anthrones are readily oxidised in presence of alkali. The alkalinity of alumina is evidently sufficient to bring about this change in some cases. Cahn and Simonsen[10] have shown that aloe-emodin-anthranol is converted to aloe-emodin by aerial oxidation in alkaline solution.

Conclusions

1. Alumina is too strong an adsorbent for use with hydroxyanthraquinone derivatives, probably due to the formation of lakes.
2. Heavy magnesium carbonate gives more promising results. The fact that it dissolves readily in dilute acids, thus liberating the adsorbed substances, which may be extracted by an immiscible solvent, is very useful. Its alkalinity is a disadvantage, and may lead to decomposition of the adsorbate.
3. Acetylation of the hydroxyanthraquinone derivatives before chromatography reduces the extent to which they are adsorbed, and so makes elution easier.
4. Atmospheric oxidation of anthrones to anthraquinones may take place in presence of alkali, and may occur on a chromatographic column.

The author is greatly indebted to Dr. Neil Campbell, F.R.S.E., for advice and encouragement while carrying out the above work.

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DISCUSSION

The paper was read by Dr. G. H. Macmorran.

The Chairman said that it would be interesting to know whether the author had anything to say about the pharmacological activity of the various adsorbates.

DR. J. W. FAIRBAIRN (London) said that he could confirm the author's findings, that alumina was far too strong, and the best adsorbent he had tried was magnesium carbonate. Figure 1 showed a column which was red at the top, but in the paper itself there was no mention of anthraquinones being contained in that column. That might be due to a misprint because later it was stated that chloroform did extract free anthraquinones, and therefore they should appear on the column. In Figure 4, the pale pink and deep pink bands gave no reaction with the anthraquinones. Was it possible to elute the anthraquinones, and should one resort to treatment with acid and extraction with chloroform? Although ethyl alcohol extracted a certain amount of glycosides, it was a very poor solvent for the purpose; one could not hope to get all the glycosides of senna out with ethyl alcohol.

DR. G. H. MACMORRAN, replying, said that he had done no work on the pharmacological activity of any of the zones on the chromatographic columns. The top zone of Figure 1 gave no reaction for combined anthraquinones, and that was to be expected, but he thought that it did give a reaction for free anthraquinones. With regard to Figures 4 and 5, he did not think that there were any reactions given for free anthraquinones. In the paper there was a table showing that the first three zones gave reactions with Bornträger's test, indicating the presence of free anthraquinones, and zones 2, 3, 4 and 5 gave a positive reaction with Molisch's test, so that only zones 2 and 3 gave reactions for both the anthraquinones and the carbohydrates. It was suggested that some of the glycosides had been hydrolysed on the column and the sugars carried further down the column, and therefore in zones 4 and 5 there was a positive reaction with Molisch's test but not for the anthraquinones.