A Paper Chromatographic Study of Aloe, Aloin and of Cascara Sagrada*

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A qualitative and quantitative study of aloe, aloin, and cascara sagrada by the use of paper chromatography was undertaken. The results indicated the presence of aloe-emodin and anthranol in the free state and in glycosidal combination in all five of the drugs investigated. In addition, the Curaçao aloe contained chrysophanic acid and the cascara sagrada contained chrysophanic acid and emodin in their free state and also in glycosidal combination.

A LITERATURE REVIEW of aloe indicated that there is still considerable question with regard to its chemical composition. In addition, it was found that the work that appeared in the literature was conducted almost entirely on one official species of aloe (Curaçao). This investigation was undertaken to ascertain the major constituents present in the three official species of aloe, and to quantitate, if possible, the active constituents present, utilizing the technique of paper chromatography.

Since aloin is prepared from aloe a study of a sample of aloin was included in this investigation. Cascara sagrada having similar type major constituents was also included in the investigation with the hope that paper chromatography would shed new light on the constituents. As far as the authors could determine, paper chromatography has never been employed in the analysis of these drugs. Paper chromatography, however, has been applied by Shibata and Takido (1) for the qualitative and quantitative studies of several varieties of rhubarb.

QUALITATIVE STUDIES

Preparation of Authentic Specimens of Some Anthracene Derivatives.—The nature of the investigation undertaken necessitated that pure samples of some of the anthracene derivatives be available. Accordingly, the following compounds were prepared: aloe-emodin, aloe-emodin anthranol, chrysophanic acid, and emodin.

Aloe-emodin was prepared according to the method of Cahn and Simonsen (4). Aloe-emodin anthranol was prepared according to Hauser's method (5). Chrysophanic acid and emodin were prepared by the processes of John H. Gardner (6, 7). The pure compounds were identified by their melting points and by a study of their absorption curves.

Spectrophotometric Examination of the Prepared Anthracene Derivatives.—The maximum absorption of a substance is an important criterion for its identification. It also provides a means of quantitating the substance under investigation. Therefore, the transmittance curves of the pure anthracene derivatives prepared previously were determined. The results illustrated the points of maximum absorption of aloe-emodin and chrysophanic acid at 430 mμ, emodin at 440 mμ, and aloe-emodin anthranol at 360 mμ.

Identification of Constituents

(a) Extraction.—Chloroform extracts of the free and the combined (glycosidal combination) anthracene derivatives from verified powdered specimens of Cape aloe, Socotrine aloe, Curaçao aloe, aloin, and cascara sagrada were prepared by Daels' method (8) as modified by Brody, Voigt and Maher (2), using smaller volumes of the extracting menstruum (25 ml chloroform per 5 Gm. of drug).

(b) Paper chromatography.—The method used for the analysis of the drug extracts was basically that of Shibata and Takido (1). Several strips of chromatographic paper, Whatman No. 1, 60 cm. long and 4 cm. wide, were prepared. The test solutions in chloroform containing the free and combined anthracene derivatives prepared above were spotted with a micro-pipet on a start line which was drawn at a 4-cm. distance from one of the ends of each of the strips. Volumes of the chloroform solutions ranging from 100–250 λ were employed. The strips were then allowed to air-dry before chromatographing them.

The chromatography was carried out by the ascending method using petroleum ether (b. p. 65–110°) saturated with 97 per cent methyl alcohol at room temperature. The bottom of a chromatographic jar (30 cm. in diameter and 60 cm. in length) was covered to a depth of two inches with the above solvent. The chamber was closed tightly and left for about 6 hours to ensure atmospheric saturation.

The strips containing the test solution were stapled on a grid made of five glass rods. The ends of the strips bearing the test solution were the farthest from the rods. A maximum of 20 paper strips could be hung on the grid at each chromatographic run. The strips were transferred to the solvent saturated chromatographic chamber which was then completely sealed to maintain the atmospheric equilibrium. The chromatograms were allowed to develop for 10-12 hours.

The strips were removed from the chromatographic jar and the solvent fronts marked. After drying, the developed paper strips were sprayed with 0.5 per cent methanolic magnesium acetate solution; the strips then were heated at 100° for

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sagrada contained chrysophanic acid and emodin in their free state and also in glycosidal combination.

2. Iso emodin previously reported present in Curaçao aloe and cascara sagrada was not detected in either of these two drugs.

3. Paper chromatography was utilized in the quantitative evaluation of the three official species of aloe. The results showed the superiority of the Curaçao aloe to either the Cape or the Socotrine aloes.

REFERENCES

(1) Shibata, S., and Takido, M., *J. Pharm. Soc. Japan*, **72**, 2789(1950).
(2) Brody, T. M., Voigt, R. F., and Maher, F. T., THIS JOURNAL, **39**, 666(1950).
(3) Gibson, M. R., and Schwarting, A. E., *ibid.*, **37**, 206 (1948).
(4) Cahn, R. S., and Simonsen, J. L., *J. Chem. Soc.*, 2573 (1932).
(5) Hauser, F., *Pharm. Acta Helv.*, **6**, 79(1931).
(6) Gardner, J. H., THIS JOURNAL, **23**, 1178(1934).
(7) Gardner, J. H., *ibid.*, **28**, 143(1939).
(8) Daels, M. F., *Bull. acad. roy. méd. Belg.*, **29**, 779 (1929).
(9) Thorpe, T. E., and Miller, A. K., *J. Chem. Soc.*, **61**, 1(1892).
(10) Schwabe, P., *Arch. Pharm.*, **226**, 569(1888).
(11) Jowett, H. A. D., *Proc. Am. Pharm. Assoc.*, **52**, 288 (1904).
(12) Green, M. W., King, C. G., and Beal, G. D., THIS JOURNAL, **27**, 95(1938).
(13) Keimatsu, S., and Hirano, I., *J. Pharm. Soc. Japan*, **51**, 230(1931).
(14) Le Prince, M., *Compt. rend.*, **129**, 606(1899).
(15) Liddell, R. W., King, C. G., and Beal, G. D., THIS JOURNAL, **31**, 161(1942).
(16) Fairbairn, J. W., *Pharm. J.*, **148**, 198(1942).
(17) "The Pharmacopeia of the United States", 14th rev., Mack Publishing Co., Easton, Pa., 1950, pp. 26, 777, and 780.

A Simplified Assay of Nux Vomica Tincture*

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The time of the N. F. assay has been materially shortened by employing a preliminary isolation procedure involving adsorption on aluminum oxide followed by elution of the alkaloids with 70 per cent alcohol. The eluate is then subjected to ion exchange on Amberlite* IRC 50 and subsequent elution with 0.1 *N* HCl in 70 per cent alcohol. Final determination of strychnine and brucine, is made spectrophotometrically at 255 and 264 mμ. A batchwise ion exchange technique has been developed which has been found to be simpler and more rapid than the conventional columnar technique.

THE OFFICIAL ASSAY for strychnine in nux vomica galenicals is of good precision but relatively time consuming. The lengthy nature of the determination is due for the most part to difficulties encountered in the extraction of the strychnine and in the elimination of the brucine which always accompanies the strychnine in such preparations.

Numerous investigations have been conducted with the object of improving the official method of assay (1–3). Chromatographic procedures have been studied (4, 5) and the chromatographic separation of strychnine and brucine has been reported by La Rocca and Burlage (6) and others (7, 8). El Ridi and Khalifa (9) described a spectrophotometric assay which employed a preliminary purification of the alkaloids by adsorption of the galenical on alumina, followed by elution of the alkaloids with ethanol. Spectrophotometric methods for the simultaneous determination of strychnine and brucine have been reported (10, 11), and Bhattacharya and Ganguly (12) examined the deviations from Beer's law which may occur during such determinations. Analyses based on ion exchange techniques have also been described (13, 14). Recently, Piantadosi (15) suggested an improved assay based on the use of cation exchange resins. This procedure offered several advantages but the method still entailed the removal of brucine via oxidation followed by a tedious extraction of the strychnine.

In reviewing the official assay method and the work of others, it became apparent that a need still existed for a more rapid assay for strychnine in nux vomica galenicals. The present work describes such an assay for strychnine in nux vomica tincture, and it is believed that the method can be easily extended to include the assay of other nux vomica galenicals.

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5 minutes resulting in a distinct orange, pink or yellow coloration.

Paper strips on which were spotted the pure authentic anthracene derivatives previously prepared were chromatographed simultaneously with the strips containing the test solution to indicate the exact R_f values. In addition, the comparison of the transmittance curves of the alcohol-eluted compounds from the unknown chromatograms with the transmittance curves of the compounds eluted from the known chromatograms served as a further criterion for identification.

Table I illustrates the R_f values of the spots that appeared and the color they gave with the methanolic magnesium acetate solution. Table II contains the qualitative results of the anthracene derivatives found in the samples studied.

TABLE I. R_f VALUES OF ANTHRACENE COMPOUNDS AND THE COLOR OF THESE CIMPOUNDS WITH THE MAGNESIUM ACETATE REAGENT

Compound	R_f Value	Color of Compound with Methanolic Magnesium Acetate Reagent
Anthranol	0.09	Yellow
Aloe-emodin	0.25	Orange, becoming pink
Emodin	0.44	Orange, becoming pink
Chrysophanic acid	0.94	Orange, becoming pink

TABLE II. ANTHRACENE DERIVATIVES FOUND IN THE SAMPLES STUDIED

Anthracene Derivative	Curaçao aloe	Cape aloe	Socotrine aloe	Aloin	Cascara sagrada
Anthranol	+	+	+	+	+
Aloe-emodin	+	+	+	+	+
Chrysophanic acid	+	−	−	−	+
Emodin	−	−	−	−	+

DISCUSSION

As indicated in Table II the experiments conducted on Curaçao aloe revealed the presence of anthranol, aloe-emodin, and chrysophanic acid in both the free state and in glycosidal combination. The anthranol was identified on the basis that it conformed to previously reported characteristics in that: (*a*) it exhibited no absorption peak at any wavelength over a wide range of the ultraviolet and visible portions of the spectrum; (*b*) it gave a yellow color with alkalies instead of the orange or pinkish coloration characteristic of the other anthracene derivatives. This yellow coloration was also noticed by Brody, Voigt and Maher (2) and Gibson and Schwarting (3) who employed an alkaline chromatographic column for separating the anthracene derivatives of Curaçao aloe and cascara sagrada, respectively. These results of the Curaçao aloe differ from that of Brody, Voigt and Maher (2) in that chrysophanic acid was isolated and iso-emodin was not, while Brody, Voigt and Maher isolated iso-emodin and not chrysophanic acid.

In the analysis of cascara sagrada, the anthracene derivatives emodin, aloe-emodin, chrysophanic acid, and anthranol were detected as indicated in Table II. This differs from the results of Gibson and Schwarting (3) in that chrysophanic acid was detected and iso-emodin was not, while Gibson and Schwarting isolated iso-emodin and not chrysophanic acid. Le Prince (14) and Liddell, King and Beal (12, 15) have reported the presence of chrysophanic acid in cascara sagrada. Jowett (11), in addition to Gibson and Schwarting, did not report the presence of chrysophanic acid in cascara sagrada.

One might at first believe that these two compounds, iso-emodin and chrysophanic acid, have been confused. However, evidence of their different physical properties shows that not to be the case. In this experimental work the identity of chrysophanic acid was established by the following: First, it was soluble in ammonia giving a positive Borntrager reaction (16) (iso-emodin does not dissolve in ammonia and thus gives a negative Borntrager reaction). Second, it had the same absorption maximum as pure chrysophanic acid. Third, it gave an R_f value identical with the R_f value of a known pure sample of chrysophanic acid having a melting point of 196°. This R_f value agreed closely with the R_f value of chrysophanic acid prepared and chromatographed by Shibata and Takido (1). These two investigators found that anthraquinone compounds having two hydroxyl groups and one methyl group in their anthracene nucleus such as chrysophanic acid and its isomers usually yielded a very high R_f value (0.92). The introduction of a third hydroxyl group into the anthracene nucleus as in emodin and iso-emodin gave a much lower R_f value (0.50).

The presence of only anthranol and aloe-emodin was detected in the free state and in glycosidal combination in the Cape and Socotrine aloes. No chrysophanic acid could be detected on the chromatograms of their extracts. An unidentified constituent having a slightly higher R_f value than anthranol and an absorption maximum at 430 mμ was detected in the free state in Socotrine aloe. It was thought, at first, that some tailing of the aloe-emodin spot occurred, but repeated experiments under the same conditions revealed the separate existence of this extra spot.

The aloin sample used was found to contain only anthranol and aloe-emodin. The chemical composition of aloin differs according to its commercial source (17). Since no chrysophanic acid could be spotted on the chromatograms of its extracts, it was concluded that the aloin sample was not obtained from Curaçao aloe.

At no time in any of the drugs studied could aloe-emodin anthranol, first described by Hauser (5), be detected on the chromatograms. The pure aloe-emodin anthranol prepared according to Hauser's method (5) yielded two components when chromatographed. One of these components was aloe-emodin and the other component was identical with the anthranol present in all the drugs investigated here. It is possible, therefore, that the compound of Hauser is not a single entity but rather a two component substance.

QUANTITATIVE STUDIES

A comparative quantitative study of the official kinds of aloe was undertaken after separation of the constituents by the use of paper chromatography was achieved.

Moisture Determination.—The moisture content was determined by the U. S. P. XIV method (17) for drugs containing no volatile principles at 100°. The purpose of performing the moisture determination was to obtain data so that succeeding calculations could be made on a dry weight basis.

Determination of Water-Soluble Extractive. The amount of water soluble extractive was determined by the U. S. P. XIV method (17). The results appear in Table III.

TABLE III. PERCENTAGE OF WATER-SOLUBLE EXTRACTIVE OF ALOE

Aloe	Per cent of Water-soluble extractive
Curaçao	80.89
Cape	70.04
Socotrine	41.95

Per cent Recovery of Anthracene Compounds from Chromatograms. It has been reported in the literature that in quantitative practice, compounds separated by paper chromatography are not recovered completely from the developed chromatograms. Whether this was also true of the anthracene compounds was not known.

A 200 λ volume of a one per cent alcohol solution of each of aloe-emodin and chrysophanic acid was spotted on the starting line of a number of chromatographic strips and was chromatographed ascendingly for 10-12 hours. After drying, the strips were eluted with alcohol in a desiccator overnight. The eluents containing the anthracene compounds were diluted to a 10-ml. volume, their transmittance determined spectrophotometrically and the values interpreted from a calibration curve plotted previously on 2 x 10 semilogarithmic paper.

The results indicated that recovery was not complete. The average percentage recovery was 88.5 of aloe-emodin and 86.9 of chrysophanic acid. No satisfactory explanation has been reached which accounts for the loss on elution.

Quantitative Determination of the Measurable Anthracene Derivatives of Aloe.—Chloroform extracts of the anthracene derivatives of aloe present in the free state as well as in glycosidal combination were prepared separately by the method previously described. Depending on the color of the extracts, volumes ranging from 100-500 λ were spotted on paper strips and chromatographed ascendingly as previously described. After establishing the identity of the spots, they were eluted with ethyl alcohol, their transmittance read on a Beckman spectrophotometer, Model DU and their concentration determined from the standard curves previously plotted on 2 x 10 semilogarithmic paper taking into consideration the moisture content of the powdered drugs and the percentage of the compounds recovered on elution.

Only the anthracene compounds having an absorption maximum could be evaluated. These compounds included aloe-emodin and chrysophanic acid. The results of the analysis of all the aloes appear in Table IV.

The total anthracene content could not be determined since anthranol, a compound present in all aloes in the free as well as the combined state, could not be quantitatively determined by the same spectrophotometric method used for the determination of the other constituents, as no single wavelength could be chosen for its analytical determination.

TABLE IV. QUANTITATIVE STUDIES OF ALOE

Sample	Curaçao aloe	Cape aloe	Socotrine aloe
Free Aloe-emodin expressed in mg. %	22.15	6.70	6.26
Combined Aloe-emodin expressed in mg. %[a]	149.73	60.12	37.62
Free Chrysophanic acid expressed in mg. %	7.81	...	...
Combined Chrysophanic acid expressed in mg. %[a]	33.77	...	...

[a] Combined refers to that occurring in glycosidal combination.

DISCUSSION

The results of the detemination of the water-soluble extractive (Table III) by the U. S. P. XIV method (17) showed that the Curaçao aloe contained more water-soluble extractive than the Cape aloe and Socotrine aloe. The difference in the amount of water-soluble extractives is not as great or significant as the difference in the amount of the anthracene derivatives. The data in Table IV show that there was considerable difference in the aloe-emodin content in the three varieties of aloe investigated. The Curaçao aloe was found to be the richest in the amount of aloe-emodin present. It contained approximately 2.5 times as much aloe-emodin as the Cape aloe and 4 times as much aloe-emodin as the Socotrine aloe. In addition, the Curaçao aloe contained a considerable amount of chrysophanic acid. It is concluded, therefore, that the Curaçao aloe was superior in quality to either the Cape or Socotrine aloes.

The data in Table IV reveal also that the free extracts of aloe contained far less anthracene compounds than the combined extracts. In the Curaçao aloe, approximately one-seventh of the aloe-emodin and one-fourth of the chrysophanic acid were found in the free state. In the Cape aloe one-ninth of the aloe-emodin occurred free and in the Socotrine aloe approximately one-sixth of the aloe-emodin was in the free state.

SUMMARY

1. The constituents of Curaçao aloe, Cape aloe, Socotrine aloe, aloin, and cascara sagrada were successfully separated by the technique of ascending paper chromatography, using Whatman No. 1 filter paper and petroleum ether (b. p. 65-110°) saturated with 97 per cent methyl alcohol as the solvent. The results indicated the presence of aloe-emodin and anthranol in the free state and in glycosidal combination in all five of the drugs investigated. In addition, the Curaçoa aloe contained chrysophanic acid and the cascara