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A COMPARATIVE INVESTIGATION OF METHODS USED TO ESTIMATE ALOIN AND RELATED COMPOUNDS IN ALOES

By McCarthy, T. J., and R. K. Mapp

Numerous papers have been published on the determination of aloin or related hydroxyanthraquinones in aloes and other anthraquinonecontaining drugs. Many of these methods have been reviewed by Kraus (1959). Apart from the extraction methods, including extraction as the calcium salt, which give low results, and the methods requiring sophisticated analytical apparatus, for example, polarography (Stone, 1947) and gaschromatography (Wang et al., 1963), it is seen that analytical methods fall into two broad groups, chromatographic and spectrophotometric. Frequently the former is used as a means of isolation of the active principle, and is followed by spectrophotometric assay.

With such a variety and number of assay methods available the important factors to be considered are analytical accuracy, reproducibility and the time taken for the analysis. The last factor is of importance not so much to the academician but to the worker in industry.

With these factors in mind it was decided to carry out comparative estimations of aloe and aloin samples using methods which conceivably would comply with the criteria elucidated above. As reference assay was used the method of the Joint Committee of Pharmacy and Analytical Chemistry (1967), (based on the method of Fairbairn and Simic (1963)), since this method had been examined conjointly by five British analytical laboratories, with close agreement. This method is hereafter referred to in the text as the Fairbairn method, and involves removal of any aloe-emodin already present by solvent extraction, and conversion of aloin (and aloinosides) to aloe-emodin by ferric chloride oxidation. Alkaline aloe-emodin solution is finally estimated spectrophotometrically.

The second method is based on the periodate oxidation of aloin recommended by Möhrle (1962), as modified by Hörhammer et al., (1963) and Böhme and Kreutzig (1965). Once again the resulting alkaline aloe-emodin is measured spectrophotometrically.

For the third method aloin was separated by thin layer chromatography according to the method of McCarthy (1968), using Silica gel G (Merck). Since the latter is slightly soluble in methanol and absorbs at 360 nm, it was found that a correction factor of 0.022 had to be deducted from the extinction readings. (This correction factor was the same for weights of silica gel from 25 mg to 100 mg per 5 ml methanol, but may vary batchwise).

In addition, pure aloin was also estimated by the method of the B.P.C. 1968, where the extinction of a 0.0025% aqueous solution at 354 nm was found to be 0.61.

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Aloin content of

Sample No.	Fairbairn
1	13.3
2	15.9
3	19.9
4	12.9
5	19.0
6	14.9
7	12.8
8	11.0
9	12.4
10	12.5
11	29.7
12	30.5

In addition to aloin, aloes may contain other anthraquinone derivatives such as isobarbaloin, aloinosides, homonataloin, betabarbaloin and aloe-emodin. These substances have also been considered in this investigation.

Materials

Aloes. Samples 1 to 10 were Cape aloes, sample 11 was Curacao aloes and sample 12 was dried aloes collected from *A. africana* × *A. ferox*, and contained aloinosides.

Aloin was donated by MacFarlan, Smith Ltd., Edinburgh.

Homonataloin was extracted from *A. speciosa*, as per Haynes et al. (1960).

Chromatography was by T.L.C. on Silica gel G (Merck), on plates activated at 105° C for 30 minutes.

Solvent Systems:

a) Choloroform : ethanol (95%) (3 : 1)

b) Ethyl acetate : glacial acetic acid : water (5 : 1 : 4)

Spectrophotometry. All readings were made in 1 cm quartz cells, using a Beckman D. B. spectrophotometer.

Results

Aloin

Reference to table 1 indicates the percentage of aloin in each of the twelve samples analysed. As will be seen, in many cases there is close agreement between the first three methods tabulated, but the fourth method (Möhrle, modified by Hörhammer et al.) gives consistently high figures.

Table 1

Aloin content of aloes by different methods (%)

Sample No.	Fairbairn	T.L.C.	Möhrle modified by Böhme and Kreutzig	Möhrle modified by Hörhammer et al.
1	13.3	12.5	13.3	15.7
2	15.9	15.3	14.3	19.3
3	19.9	20.5	19.0	23.9
4	12.9	12.2	11.5	16.0
5	19.0	19.1	19.0	22.8
6	14.9	14.4	14.8	19.4
7	12.8	13.2	8.5	15.7
8	11.0	10.5	9.7	15.0
9	12.4	11.7	12.0	15.0
10	12.5	11.5	12.0	16.3
11	29.7	29.8	29.0	34.6
12	30.5	29.9	27.4	34.0

The reason for this error, (which has been overcome by the modification of Böhme and Kreutzig), is the use of water as a blank instead of aloe plus ammonia, which gives a yellow colour due to resinous matter present. However, this use of ammonia plus sample as blank has a disadvantage in the assay of aloin of high purity where little or no resinous matter is present, since aloin itself gives a deep yellow colour with ammonia, whereas the aloin converted by periodate to aloe-emodin gives with ammonia a red colour. Thus the aloin/ammonia blank results in a lower reading, as will be seen by reference to table 2, where an approximately 10% lower figure was obtained by the Böhme and Kreutzig modification.

Comparison of the aloin content of ten Cape aloe samples (as determined by the Fairbairn method) with their water-soluble extractives (as per B.P. 1968 shows (table 3) that a crude relationship exists between the two, but the varying proportion of water-soluble resins precludes the use of this relationship for accurate work.

Table 2

Percentage purity of aloin samples

Aloin (moist) (two samples)		Spectrophotometric (B.P.C.)	T.L.C. (two workers) A	Möhrle (Böhme and Kreutzig) B	$\frac{B}{A}$ %
1)	92.8	–	92.5 & 92.7	83.5	90%
2)	90.7	90.7	90.3 & 90.3	81.3	89.6%

Table 3

Water-soluble extractive compared with aloin content

Sample	Water-soluble extractive % (W.S.E.)	$\frac{W.S.E.}{5}$	Aloin %
A	76.0	15.2	13.4
B	75.0	15.0	15.9
C	83.0	16.6	19.9
D	63.2	12.6	12.9
E	78.4	15.7	19.0
F	77.0	15.4	14.9
G	59.6	11.9	12.8
H	56.6	11.3	11.0
I	72.5	14.5	12.4
J	64.6	12.9	12.5

Isobarbaloin

Since isobarbaloin is an optical isomer of aloin, which on hydrolysis yields aloe-emodin (Gardner and Joseph, 1937) the methods of Fairbairn and

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Möhrle (Böhme and Kreutzig) B	$\frac{B}{A}$ %
83.5	90%
81.3	89.6%

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Aloin %
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Möhrle (as modified) detect this compound quantitatively as aloin. Since assay aloin and isobarbaloin have the same spectral peaks, the latter is assayable by the T.L.C. method, but with slightly less accuracy than the preceding methods, as will be seen from table 4. In solvent a) aloin and isobarbaloin run as a combined spot, whereas in solvent b) they are separated, and each is visible under ultra violet light (long wave). The sample used was Curacao aloes.

Table 4

Method	Aloin plus isobarb.	Aloin.	Isobarb.
T.L.C.	Solvent a) 29.8%		
	Solvent b) 28.25%	15.75%	12.50%
Fairbairn	29.66%	not applicable	
Möhrle (B and K)	29.03%	not applicable	

Aloinosides

In similar manner aloin and aloinosides are estimated conjointly by the Fairbairn, and the Böhme and Kreutzig methods, but in Solvent a), using T.L.C., they may be estimated separately. This could be a possible disadvantage, in that an inexperienced worker running aloin as a control on T.L.C. plates could overlook the aloinosides, which have a purgative activity equal to aloin, (Hörhammer et al., 1963) and which run at a lower R_F. Estimating the aloinoside content on the aloin graph gave figures for this sample (sample 12, table 1) of 15.64% aloinoside and 14.25% aloin (total 29.89% as aloin), whereas the Fairbairn, and the Böhme and Kreutzig modification gave, respectively, 30.5% and 27.4%, as aloin. Possibly the lower reading of the latter is due to the relatively short oxidation period, since the aloinoside must first be converted to aloin.

Homonataloin

Homonataloin can be estimated by T.L.C. (McCarthy, 1968) in a manner analogous to aloin. Aloes containing homonataloin rarely appear on the market since the decline of the Natal aloes industry, but since some 25 South African species contain homonataloin (McCarthy, 1969), these could easily be included in error by unskilled collectors. It was wondered what effect this would have on the methods of Fairbairn, and Böhme and Kreutzig.

The former method employs a ratio of extinctions at 500 nm/440 nm to exclude impurities. If this ratio is less than 1.9 the result is rejected. This was found to be the case with homonataloin, which has a peak in NaOH at 460 nm. Furthermore a mauve coloured solution, not red as for aloin, is obtained with homonataloin in sodium hydroxide solution.

The Böhme and Kreutzig modification, however, has no such limiting ratio and the solution, although having its maximum at 460 nm, nevertheless gave a high reading at 510 nm, the wavelength recommended by these authors for aloin determination. The homonataloin solution is orange in contrast to the red colour of aloin, but many workers are colour-blind, and thus an extinction ratio at 510 nm/460 nm of greater than one should be introduced to eliminate any possible error. From the point of view of purgative activity the presence of homonataloin raises problems, since Mapp (1969) has shown homonataloin to have a purgative activity in rats equal to aloin. However, the reading at 510 nm would be lower than for aloin, of equivalent content, and consequently the quantity of aloe used would be greater if homonataloin were the only active principle, as for example if collected from *A. marlothii*, which resembles *A. ferox*, and which Van Oudtshoorn (1965) has shown may contain either aloin or homonataloin depending upon its locale. This could result in excessive purgation.

Beta-barbaloin

Beta-barbaloin is listed by the B.P.C. 1968 as one of the constituents of aloes. According to Denston (1951) it is an amorphous, water-soluble compound, isomeric with barbaloin and formed from it upon heating at high temperature. Cape aloes contains most (about 8%), due to prolonged boiling of the juice and the high temperature attained towards the end of boiling.

Consequently aloin was taken and heated at 170° C for 3 hours. This caused the aloin to blacken, and a portion became methanol-insoluble. The methanolic solution, when chromatographed by T.L.C. using solvent a), showed two slightly darker orange spots above the aloin. These spots reacted the same as aloin when sprayed separately with methanolic magnesium acetate 0.5%, and ammonia 10% and were unreactive to aqueous periodate and Fast blue B. Methanolic solution of each new spot had no peak at 359 nm (in contrast to aloin), with the main peak at 295 nm and a small peak at 252 nm. In solvent b) only one spot, slightly above and merging with the aloin, was formed, which within a few days was visible in daylight as a light brow spot.

This methanolic solution was then added to a methanolic solution of aloes, and chromatographed on T.L.C. using solvent a). These spots, presumed to be beta-barbaloin, were completely obscured by the resinous constituents of aloes, and would thus not be visible in the T.L.C. assay.

Destruction of aloin by heating

Aloin was heated at 150° C for 2 hours and then assayed by T.L.C. and the Fairbairn methods, giving, respectively 84.0% and 84.5% aloin. The aloin used was pure aloin containing 7.2% moisture, and thus should have assayed at 92.8% purity, indicating a loss on heating of circa 8%.

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Figures for the heating of aloe juice in varying thicknesses and for varying times are given in tables 5 and 6 (McCarthy, 1964), where the destructive effect of high temperatures and thick layers (and consequently longer times) are evident. From these figures it can be estimated that by the conventional heating method on wood fires, some 12,000 Kg of aloin is destroyed annually in South Africa alone, since some 500,000 Kg of aloes is exported annually (Ramstad, 1959).

Table 5
Aloin loss (%) on drying aloe juice

Drying time a) at 150° C	Layer thickness	Original % aloin w/w	Final % aloin w/w	Actual aloin loss %	Relative aloin loss %
1 hr. 25 min	1 cm	15.7	13.9	1.8	11.4
2 hours	1½ cm	24.6	21.4	3.2	13.0
2 hrs. 25 min	2 cm	15.7	13.5	2.2	14.0
22 min	1–2 mm	24.6	23.6	1.0	4.1
15 min	1–2 mm	21.9	20.4	1.5	6.8
b) at 125° C					
15 min	1–2 mm	21.9	20.2	1.7	7.7

Table 6
Relative aloin loss in aloe juice by different heating methods

	Heating method	Relative aloin loss
1)	Over wood fires (conventional)	8.3 – 17.9%
2)	Sun dried on canvas	5.9 – 9.5%
3)	Electric oven a 150° C	
	a) thick layer	11.4 – 13%
	b) thin layer	4.1 – 7.7%
4)	Hot air (tablet coater)	2.2%
5)	Rotary drum drier a 110° C	0 – 0.4%

Aloe-emodin

When the results quoted in tables 5 and 6 were first obtained it was thought that a large proportion of the aloin destroyed by heating had been broken down to aloe-emodin, since this compound became evident chromatographically. However, it was the experience of the Joint Committee of the Pharmaceutical and Analytical Chemical Societies that free aloe-emodin and O-glycosides were present in aloes in amounts of less than 0.5%. At first we thought this figure to be too low, but selective extraction of aqueous aloe samples with carbon tetrachloride gave figures for aloe-emodin in a sample of Curacao and in three samples of Cape aloes as 0.29%, 0.23%, 0.162% and 0.125% respectively, confirming their observation

The Joint Committee hence state that the preliminary treatment set out in Appendix I of their assay method to eliminate free aloe-emodin and 0-glycosides could be omitted without marked influence on the final results. This would normally be true, but should aloinosides be present to the extent of our sample No. 12 (table 1) then obviously differences would result.

Periodate-Positive Compound

Böhme and Kreutzig (1963) described a compound in Cape aloes which formed a purplish colour on spraying with aqueous periodate, which rapidly faded. This compound was observed in only one sample (sample 5, table 1), and appears on or just below aloin in solvent a) on T.L.C. This compound, which from the analytical results is not reflected in the assay systems, nevertheless has a marked purgative effect on both rats and humans, as will be reported in a further paper. Unfortunately too little of the sample was obtained to allow extraction of this compound.

Discussion

Having investigated four analytical methods for aloes and aloin we have shown (table 1) that close agreement exists between the results of three of these.

In table 7 are listed the working times for these, where it will be seen that the thin layer method requires the least time, either in actual manipulative time or in overall time. This time can be reduced by using pre-heated plates.

Table 7

	Fairbairn	Möhrle (Band K)	T.L.C.
Total time	6.5 hours	2.75 hours	1.75 hours
Working time	2.25 hours	0.75 hour	0.75 hour

Admittedly the T.L.C. method requires a certain degree of technique in scraping away components surrounding the aloin spot, particularly in very resinous aloe samples. Böhme and Kreutzig (1964) have separated aloin from the periodate-positive and light blue resinous spot by using a solvent system of chloroform : ethanol (95%) : water (60 : 30 : 2). However our results (table 1) show that neither compound appears to interfere to any great extent even though frequently overlying the aloin. The higher the aloin content, the better the result using T.L.C. The converse holds true for aloin by the Böhme and Kreutzig modification due to the aloin/ammonia blank, as has been discussed. Moreover their method is described as temperature sensitive (storage is at 20° C). The method does not allow for pre-existing aloe-emodin, and is unsuitable for homonataloin and aloinosides, although these are rare. Apart from this we have found the method

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easy to use. The Fairbairn method is comparatively accurate but tedious, which is the same criticism we have for the various column chromatographic methods we have tried, including that of the D.A.B. 7.

Summary

The assay of aloin in aloes has been investigated using different methods, and these have been discussed. Furthermore, the presence of homonataloin, aloinosides and similar compounds in aloes have been investigated in relation to these assay methods. The effect of heating aloe juice in relation to aloin destruction has also been discussed.

Zusammenfassung

Die Genauigkeit, Reproduzierbarkeit und der Zeitaufwand der Bestimmungen von Aloin und verwandter Verbindungen werden nach 3 in der Literatur beschriebenen Methoden miteinander verglichen.

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