of the amount taken. Therefore, in applying this method to the assay of ergot, the last ether wash can be extracted with 1% tartaric acid and an approximation of the amount of ergometrinine can be obtained. From some preliminary results recently obtained on two different samples of ergot, the ergometrinine present amounted to about half of the ergometrine content. If this is true for all samples of ergot, the error due to ergometrinine would amount to about 2–3%.

None of the five water-insoluble alkaloids tested came through in the water extract. It is regrettable that the remaining three water-insoluble alkaloids, ergotaminine, ergocristine and ergocristinine were not available for testing. It is felt, however, that they will not interfere with the method as they are all water-insoluble, ether-soluble alkaloids.

After the water-insoluble alkaloids (no ergometrine or ergometrinine present) had been run through the ether system, the second and third ether washes were shaken out separately with 1% tartaric acid and the alkaloid determined colorimetrically. It was found that the second ether contained 5.7% of the total amount of alkaloid used at the start of the assay and the third ether contained only approximately 0.2%. This indicates that in use of the Hampshire and Page (1) technique, about 6% of the water-insoluble alkaloids may be present in the water extract. Casparis and Bullet (5) reported as much as 12% of these alkaloids carried over into the water when they used the Hampshire and Page method.

Further work is being done at the present time adapting this separation scheme to the assay of ergot, the results of which will be reported in a future paper. It is hoped that this method will result in an agreement between the chemical and biological assays for ergometrine.

The author is indebted to Mr. Geo. L. Keenan of the Microanalytical Division of The Food and Drug Administration for checking the optical crystallographic properties of the purchased ergometrine.

SUMMARY

A method for the quantitative separation of ergometrine from the water-insoluble ergot alkaloids as well as from its therapeutically inactive isomer, ergometrinine, has been presented.

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A Phytochemical Study of *Aloe vera* Leaf*

By Tom D. Rowe† and Lloyd M. Parks‡

The study reported in this paper was undertaken in connection with an attempt to isolate and characterize the principle contained in *Aloe vera* leaf which is responsible for its activity in promoting the healing of third degree x-ray reactions on white rats. Although dried aloe and its constituents have been the subject of numerous investigations, little work has been done on the fresh aloe leaf. Hence, much of the work reported herein is preliminary in nature. Most of the cursory investigations of the fresh leaf which have appeared from time to time have been of a histological and anatomical nature.

Robiquet, in 1846 (1), reported studies which have since been contradicted. Reports by Unger in 1855 (2), Gasparrini in 1863 (3) and Trecul in 1872 (4) contributed little to our chemical knowledge of the leaf. Prollius in 1884 (5) contributed perhaps the most informative report on several species of aloe, while the latest work of Chopra and Ghosh, in 1938 (6), on fresh *Aloe vera* leaf reported the presence of a trace of volatile oil, non-volatile oil, resin, gum, emodin, an anthraquinone compound, and chrysophanic acid. These constituents, plus aloin, are essentially all that have been found in previous investigations.

* Based on a portion of a thesis submitted to the Faculty of the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy by Tom D. Rowe.

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From the Laboratory of Edward Kremers.

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Most of the leaf material used in this study was obtained from the Jamaica Gardens of S. B. Penick and Company[1] and was received in shipments throughout the year 1940–1941. Details concerning these shipments appear in another report (7). The investigation was divided into four main parts, namely, preliminary tests on the whole leaf, on the rind, on the pulp, and fractionation of the pulp.

EXPERIMENTAL

Preliminary Tests and Determinations on the Whole Leaf.—The fresh leaves used in this part of the work were obtained in July and August 1937, from the General and Marine Laboratory, Hollywood, Florida, and were in good condition when received. The results are summarized as follows:

1. Moisture Content (8)—96.5%.

2. Selective solvent extraction on a sample dried by distillation with xylene. The percentage of extractive is expressed on the basis of both dried weight and fresh leaf:

	Dried Leaf, %	Fresh Leaf, %
Ether	2.6	0.11
Chloroform	1.1	0.05
Alcohol	8.6	0.38
Water	80.3	3.50

3. Pentosan content (9)—0.5% of fresh weight later work indicated that most, if not all, of the pentosans were contained in the rind.

4. Cellulose content (10)—35.6% (dry weight) or 0.96% on the basis of the fresh weight of the leaf.

5. Qualitative tests showed reducing sugars, both simple and hydrolyzable, present, phenols and tannins absent.

Preliminary Tests and Determinations on the Rind.—All of the rind used in these tests was, with the exceptions noted, obtained from Jamaica during 1941. The results are summarized as follows:

1. Moisture content, determined by drying to constant weight at 105°—for Florida leaves, 82.9%; for Jamaica leaves, 90%. (This difference is probably due to the time of year during which the leaves were cut.)

2. Ash content—for Florida leaves, 3.3%.

3. Enzymes.—Qualitative tests showed the presence of an oxidase and a catalase.

4. Pentosan content—0.55% on basis of fresh weight.

5. Sugars.—The alcohol-soluble portion of the rind was found to contain, before hydrolysis, 16.1%, and after hydrolysis, 22.2% of reducing sugars.

[1] Our thanks are due to Mr. S. B. Penick for his kind coöperation in furnishing the leaves used in this investigation.

6. Nitrogen content (11).—The dried rind contained 1.39% nitrogen, equivalent to 0.14% on the basis of the fresh rind.

7. Proteins—not present in aqueous or alkaline extracts of the fresh rind.

8. Alkaloids, tannin and aloin—not present in the fresh rind.

9. Sulfur—present in the fresh rind.

10. Phenols—a trace may be present in the fresh rind.

11. Selective solvent extraction of the dried rind.—Rind was dried at 65° for 24 hours, powdered, and extracted to exhaustion with various solvents with the following results:

Solvent	Percentage of Extractive Dry Weight, %	Fresh Weight, %
Petroleum ether	1.86	0.18
Ether	1.71	0.17
Chloroform	0.71	0.007
Alcohol	10.50	1.05
Water	30.20	3.02
2% HCl	24.60	2.46
2% KOH	20.00	2.00
Marc	5.04	0.50
Total	94.62	9.38

The aqueous extract from this portion, upon concentration to a small volume, and the addition of excess alcohol, yielded a precipitate which was dried to an easily pulverized mass and which represented 23.3% of the dried rind. This substance was slightly soluble in water, readily soluble in dilute hydrochloric acid, and insoluble in either sodium or ammonium hydroxide solutions. A saturated aqueous solution of it was slightly more viscous than water and was precipitated by alkalies. The uronic acid content of this substance determined by the method of Dickson, Otterson and Link (12) was found to be 7.34%. This, plus the above information, indicated the material to be a mucilage.

12. Carotene and vitamin A content.—Colorimetric determinations, carried out by the Department of Biochemistry, University of Wisconsin, on the fresh rind, showed the presence of 7.2 micrograms of carotene per gram. No vitamin A was found. This is a normal content of carotene for fresh green leaf materials.

13. Vitamin D content.—This assay was carried out by the laboratory of the Wisconsin Alumni Research Foundation, using the U. S. P. XI procedure (13). Dried rind was extracted with water in order to remove any cathartic principle that might have been present. The rind, thus prepared, and fed at a level of 7 Gm. (equivalent to approximately 70 Gm. of fresh rind) showed no vitamin D activity, indicating the absence of vitamin D in the rind.

14. Aloin content.—Portions of the fresh rind were macerated in alcohol for periods from two to four months, strained, and the residue then extracted with boiling alcohol. Each of these alcoholic extracts was then concentrated to near dryness and the commonly used tests for aloin and related com-

pounds applied to them (14). Tests for aloin, emodin and isobarbaloin were positive in both extracts, being only slightly positive, however, in the hot alcoholic extract.

A quantitative aloin determination on the cold alcoholic extract, by the method of Smith and Jordan (15), yielded 4.6% of aloin, equivalent to approximately 0.1% of the fresh rind. This, however, must be considered as only an approximation at best since some of the aloe forming latex had undoubtedly drained out of the leaves upon cutting and during shipping. These results, together with the previous negative test for aloin in the fresh rind, show that aloin is present only after the rind has been allowed to stand. The absence of aloin in the fresh rind is in agreement with the observation of Chopra and Ghosh (6).

Preliminary Tests and Determinations on the Pulp.—The pulp is the jelly-like, colorless material contained within the *Aloe vera* leaf and constitutes approximately two-thirds of the entire weight of the leaf. The fresh leaves used in most of this part of the work were obtained from Florida in August 1939. The results are summarized as follows:

1. pH.—Determined with a Cameron pH meter, the fresh pulp was found to have a pH of 4.7.
2. Moisture content—98.5%.
3. Ash content—0.2%, on basis of fresh weight.
4. Viscosity.—The viscosity of the juice, expressed by hand from the pulp, was approximately equal to a 30% aqueous solution of gum acacia.
5. Enzymes.—Qualitative tests showed the presence of an oxidase and an amylase.
6. Protein and starch—negligible amounts were indicated.
7. Sugars—0.1% of simple reducing sugar was found. No hydrolyzable sugars were found; however, later examination of fractions of the pulp indicated the presence of small amounts of hydrolyzable sugars.
8. Calcium oxalate.—Crystals found imbedded in large amounts in the pulp after macerating in alcohol were identified as calcium oxalate.
9. Pectin and pectase.—Qualitative tests, both chemical and microscopic, by the procedures of Carre and Haynes (16), and Carre and Horne (17), failed to reveal the presence of pectin in either the fresh pulp or any of the fractions obtained from it. Tests for pectase, by the method of Davison and Williman (18), were negative.
10. Carotene and vitamin A.—Colorimetric determinations showed the absence of both of these substances.
11. Vitamin D.—Pulp, dried at 80° for 24 hours, when fed at a level of 5 Gm. (equivalent to approximately 333 Gm. of fresh pulp), showed no vitamin D activity, indicating the absence of this vitamin in the pulp. This is contrary to the statement of MacKee (19) that the healing property of *Aloe vera* pulp in the treatment of third degree X-ray reactions is thought to be due to vitamin D.

Fractionation of the Pulp.—All of the material used in this part of the work was obtained from Jamaica in three shipments during the months of July, August and September 1940. The pulp was removed from the leaves of each shipment by cutting and scraping with a spatula. In the case of the first two shipments it was pickled in alcohol and set aside for future use. The pulp from the third shipment was examined while fresh.

Fresh Pulp.—The pulp, freed from rind, was run through a meat chopper, expressed through gauze by hand, and the expressed juice concentrated by distilling under reduced pressure at a temperature of 65–70°. The aqueous distillate, which was not examined further, possessed a pleasant aromatic odor, indicating the presence of a volatile substance. To the concentrated juice remaining in the still was added five and one-half times its volume of alcohol, resulting in the precipitation of a fine, sponge-like substance, which settled out upon standing and was filtered, the hydro-alcoholic filtrate being concentrated to dryness. From the original pulp there were thus obtained three fractions:

1. The expressed pulp, preserved by placing in alcohol.
2. The alcohol-insoluble portion of expressed juice.
3. The hydro-alcoholic filtrate, not examined further at this point.

The gum-like, alcohol insoluble portion of the expressed juice was washed with alcohol and, when dried at 65° for 24 hours, was equivalent in weight to 0.1% of the fresh whole pulp. The ash content of this substance was found to be 9.5%, and the uronic acid content, 4.7%. This fact, together with its slight solubility in water, insolubility in alcohol and other physical properties, indicated the substance to be a mucilage (20, 21).

Pulp Macerated in Alcohol.—As previously mentioned, the pulp from the first two shipments of leaves was removed and allowed to macerate in alcohol for about three months. The mixture was then strained through gauze and the solid residue expressed by hand, separating the original pulp into two fractions:

1. The hydro-alcoholic solution of expressed juice.
2. The alcohol-extracted and expressed pulp.

1. To the hydro-alcoholic solution of expressed juice was added sufficient alcohol to cause complete precipitation. The precipitate formed in two layers, one on top and one on the bottom. Since there was the possibility of their being different in nature they were collected separately and, for the most part, examined separately. They were first air-dried and then dried at 65° for 24 hours. The hydro-alcoholic filtrate remaining after the above precipitation was evaporated to near-dryness under reduced pressure, resulting in a dark brown semi-solid mass.

Results of qualitative tests on these three portions are summarized as follows:

	Alcohol-Insoluble Portion		Concentrated Hydro-Alcoholic Filtrate
	Top Portion	Bottom Portion	
1. Nitrogen (11)	+	+	−
2. Protein (22, 23)	−	−	−
3. Halogen (11)	+	−	−
4. Molisch test	+	+	+
5. Phenols	−	−	−
6. Alkaloids[a]	−	−	−
7. Calcium	+	+	+
8. Reducing sugars			
Before hydrolysis	−	−	+
After hydrolysis	+	+	+
9. Aloin and emodin	−	−	−

[a] Tannic acid, T.S., and Phosphotungstic acid, T.S., produced marked coagulations but all of the other alkaloidal reagents were negative.

Results of quantitative determinations on these three portions are summarized as follows:

	Alcohol-Insoluble Portion, %		Concentrated Hydro-Alcoholic Filtrate, %
	Top Portion	Bottom Portion	
1. Nitrogen	0.55	0.70	Not determined
2. Reducing sugars (glucose)			
Before hydrolysis	0[a]		43.95
After hydrolysis	33.96[a]		43.95
3. Pentosans	0.95	0.98	2.15
4. Crude fiber (24)	0.30[a]		Not determined

[a] The top and bottom portions were combined for these determinations since the previous tests had shown them to be the same substance.

Qualitative tests on the dark brown, semi-solid mass resulting from concentration of the *hydro-alcoholic filtrate* remaining after the above precipitation showed the absence of pentoses and the presence of a ketose sugar. When treated by the benzimidazole condensation procedure of Moore and Link (25) for the identification of aldoses the results were negative, showing the absence of an aldohexose. The formation of a phenylosazone, melting at 208°, plus the positive tests for a ketose obtained previously and the elimination of an aldohexose by the benzimidazole procedure, confirmed the presence of fructose in this residue. This fact, together with the absence of a pentose, indicated that the results obtained previously in pentosan determinations were probably due to a partial degradation of the hexose during the acid distillation of the pentosan procedure.

2. *The alcohol-extracted and expressed pulp*, after being dried and powdered, was extracted with selective solvents with the following results, the percentage of extractive being expressed on the basis of both the dry expressed pulp and the fresh pulp.

	Dry Pulp, %	Fresh Pulp, %
Ether	0.48	0.002
Alcohol	8.80	0.03
Water	26.60	0.09

The ether and alcohol extractives were too small in amount to permit further examination. The aqueous extract, upon evaporation to a small volume, and addition of excess alcohol, yielded a precipitate which, upon drying and examination, was identified as the same mucilage obtained previously from the hydro-alcoholic solution of the expressed juice. The filtrate, when tested for reducing sugars, showed the presence of a reducing substance which was not a sugar. It reduced potassium permanganate solution and appeared to contain a carbonyl group, although attempts to prepare an oxime, a semicarbazone or a phenylhydrazone were unsuccessful. Although it was impossible to isolate it in crystalline state, evidence pointed to the fact that it was probably the soluble salt of a uronic acid.

SUMMARY AND CONCLUSIONS

1. Results are given for preliminary tests on the whole leaf, rind and pulp of *Aloe vera*. The alcohol-insoluble portion of the leaf was found, from its uronic acid content, to be a mucilage. The presence of simple reducing sugars and hydrolyzable sugars was shown. Fructose was identified as the simple reducing sugar.

2. An oxidase, a catalase and an amylase were found. Pectin, alkaloids, phenols, tannins, vitamin A and vitamin D were shown to be absent. Aloin was not found in either the fresh pulp or fresh rind, but was found in small amounts in the rind which had macerated for a long time in alcohol.

3. The absence of tannin, pectin, vitamin A, vitamin D and the small amount of nitrogenous substances found in the leaf or any concentrated fraction of it, proved that the healing property of *Aloe vera* leaf is not due to any of these principles or to urea.

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Book Review

Laboratory Instruction in Biochemistry, by ISRAEL S. KLEINER and LOUIS B. DOTTI. 188 pages. $7^3/_4$ x $10^1/_2$ (loose leaf). 1940. St. Louis: C. V. Mosby Co. $1.50.

This is a collection of laboratory experiments in elementary biochemistry on individual punched sheets which can be included in a laboratory notebook. The collection is intended for medical students and includes simple directions for elementary experiments in the biochemistry of carbohydrates, fats, proteins, milk, blood, urine, metabolism, food, etc. While intended primarily for medical students, it should also be of interest to pharmacy teachers and students.—A. G. D.

Further Observations on the Use of *Aloe vera* Leaf in the Treatment of Third Degree X-Ray Reactions*

By Tom D. Rowe†, B. K. Lovell‡ and Lloyd M. Parks**

In 1940 one of us (T. D. R.) published a preliminary report on the use of fresh *Aloe vera* jell in the treatment of third degree x-ray reactions on white rats (1). Attention was called at that time to the modern use of this leaf in the treatment of such reactions in humans. It should also be pointed out that various species of *Aloe* have been employed for centuries to promote the healing of wounds and fire burns. Thus, Turner in 1568 (2) cited the use of "the herbe Aloe is to hele wounds," and Coxe in 1818 (3) referred to the use of powdered aloe "to check heamorrhagies in recent wounds." Various other references have from time to time mentioned aloe, either fresh or dry, as a healing agent. The present report deals with further observations on the use of the fresh jell, or pulp, of the leaf, as well as other portions of the leaf, in the treatment of experimentally produced third degree X-ray reactions on the skin of white rats.

The procedure followed in producing the X-ray reactions and the method of treatment in the present report are essentially the same as those described in the first report, with the following differences: rats were given a single dose of 4000 r instead of in divided doses since it was found that a single dose at this level was not toxic; ether was used for anesthesia in place of pentobarbital; control areas received no treatment of any

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