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A Chemical and Pharmacological Study of *Aloe vera* Linné.

A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

by,

Benjamin Philip Hecht

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PREFACE

The development of our knowledge of natural drugs follows a rather definite sequence of investigation,--isolation of active principles, elucidation of chemical structure, and finally synthesis. It is through these efforts that much useful information is compiled, and that many important applications are realized.

The separation of the active principle of a drug is desirable for numerous reasons. It especially permits the use of the isolated product in concentrated form, often free from the undesirable effects of other substances present. An excellent example was the isolation of insulin from the pancreas.

The determination of chemical structure, aside from its academic interest, offers a means for correlating chemical properties with physiological action. Frequently, it leads to the development of newer and better medicinal products, through slight modification of chemical structure. Many such instances may be enumerated, as for example the development of plasmochin following the clarification of the formula of quinine. Moreover, from a knowledge of the chemistry of active constituents of drugs, better methods are sometimes devised for the evaluation of drugs.

Through synthesis, absolute proof is provided for the validity of a chemical formula established for a compound. Not infrequently, synthesis

may furnish a more economical source of a drug, independent of other sources which may become scarce or unavailable, as in time of war.

It was with these general objectives in mind, that a chemical and pharmacological study of the leaf of *Aloe vera* L. was undertaken.

SCIENTIFIC NAME

In the course of a series of investigations on pharmacological and chemical aspects of Aloe vera Linne, it became desirable to identify some plant material. It was found that considerable confusion existed in the literature in regard to the identity of this aloe plant. Authors in more recent times have regarded Aloe vera Linne, as a full species, and under this species name they have in addition recognised a few varieties. However, by referring to the original publications of Linne it was found that vera was not so regarded but instead was considered as a variety of Aloe perfoliata. A survey of the literature was therefore conducted in an attempt to find some explanationfor the introduction of Aloe vera L. in present day literature.

To investigate this question regarding nomenclature, Linne's "Species Plantarum" was first consulted. The 1753 edition(1) listed the species, Aloe perfoliata, and the variety, vera. In characteristic Linnean style, the trivial name (our species name) appeared in italic at the margin of the page and was designated by a number. The variety name, vera in this instance, likewise appeared in the margin, but in Roman type, and the name did not bear a number. Linne therefore did not consider vera as a species, otherwise it would have been expressed in italic and have been assigned a number.

The 1762 edition of Linne's "Species Plantarum" was not available. How-

ever, Tschirch in his "Handbuch der Pharmacognosie"(2) referred to Aloe perfoliata vera as occurring in this edition.

Likewise, the third edition(3) listed vera only as a variety, and here again vera appeared at the margin of the page and of a different type than that used in referring to species names. The chance for mistaking this variety name for a full species name is quite understandable. At least this offers a very plausible explanation and would account for the probable introduction of A. vera in present day literature. As early as 1861 Baker(4) published a synopsis of Aloineae in which he referred to A. vera, Linné in "Species Plantarum" edition I. This reference by Baker may indeed have been the origin by which vera later became to be regarded by botanists as a species.

The 1799 edition by Wildenow(5) presented a slight change. The name Aloe perfoliata vera was given; and in this publication vera was not set out in the margin. While later editions of some of Linne's works as "Systema Vegetabilium"(6), continued to record vera as a variety of A. perfoliata.

It is therefore apparent that Linné never published the name Aloe vera, except as a variety of A. perfoliata; nor is it a hyponym for the same. As a matter of fact, no botanist ever published the name A. vera for the same plant that Lamarck(7) called A. vulgaris. Miller(8) and Webb and Berthelot(9) probably referred to another plant with the name A. vera.

Nevertheless, Berger(10), in Engler's "Pflanzenreich," considered vera as a species of Aloe, and recognized three varieties under Aloe vera. The

differentiation is made largely on the color of the flowers.

var. 1. *officinalis* (Forsk.) Baker

var. 2. *chinensis*

var. 3. *Lansae* Berger

LINNEAN AND OTHER NAMES OF ALOE VERA L.*

Aloe vulgaris Houb.(11)	1596
Aloe perfoliata vera L.(12) A. foliis spinosis confertis dentatis vaginantibus planis maculatis.	1753
Aloe perfoliata // vera L.(13)	1762
Aloe perfoliata vera L.(14)	1764
Aloe vulgaris Haworth(15)	1764
Aloe barbadensis Mill.(16)	1768
Aloe officinalis Forsk.(17)	1775
Aloe vulgaris Lamarck(18)	1783
Aloe elongata Murr.(19)	1789
Aloe perfoliata λ vera L.(20)	1799
Aloe flava Pers.(21)	1806
Aloe indica Royle(22)	1839
Aloe littoralis Koen.(23)	1880 ?

*Compiled from "Index Kewensis."

DESCRIPTION OF PLANT

Aloe vera L. was one of the first aloe plants that was definitely characterized by writers on natural history. This plant was described by Britton in "Botany of Porto Rico and the Virgin Islands"(24). It is a perennial xerophytic plant, acaulescent or nearly so, and in its habits stoloniferous. The leaves are arranged more or less in rosette fashion. These are narrowly lanceolate, long acuminate, turgid, and of a pale-glaucous green, often spotted with white. When full grown, they attain a length of 3-6 decimeters. The marginal spiny teeth are paler in color and are arranged 2 centimeters apart or less. The scape is rather stout, 6-12 decimeters high, and bears distant, broad, acute scales. The inflorescence is a dense raceme, 1-3 decimeters long. Bracts, also present, are lanceolate to ovate-acute, and longer than the short pedicels. The yellow flowers are about 2.5 centimeters long, with the stamens about as long as the perianth, and the style longer.

Histologically, the leaf is on the centric type, showing a cutinized epidermis. Beneath this appears the mesophyl, the outer area of which is composed of thick walled cells, while the inner area exhibits very large mucilage cells. When the leaf is split open, this inner mesophyl has the appearance of a gel. Between these two regions, outer and inner mesophyl, fibrovascular bundles may be found. According to Youngken(25), the cells immediately located around the bundles contain the so-called "aloe-juice."

HABITAT (4)

Aloe vera L. belongs to the Mediterranean region. However, it has been disseminated throughout the world where it is found in many warm countries, as Florida, Hawaii, Porto Rico, and other West Indies.

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THE VALUE OF ALOE LEAF IN TREATMENT OF THIRD DEGREE ROENTGEN REACTIONS.

INTRODUCTION

The value of X-ray in the control of cancer and in the treatment of certain skin diseases is well known. However, its main drawback is the adverse consequences, resulting in third degree burns which interfere with any further treatment by X-ray. No method is known at the present time for preventing these occurrences; nor is treatment more successful. Various antiseptics have been applied in the treatment of such burns, but the value of these agents is limited. To be sure they prevent or reduce infection; otherwise they have no effect in altering the course of the reaction.

In recent years the fresh gel of the leaf of Aloe vera L. has been introduced as a remedy for X-ray burns. Its use was first suggested by Drs. Collins and Collins(1) who reported good results on a single case. Since then other case histories have been described (2, 3, 4, 5, 6, 7), these limited to but a few patients. The work of Rowe et al. (8,9) was the first attempt to demonstrate the value of Aloe gel, using rats as test animals for both the treatment and the controls.

The results of Rowe's experimental investigation indicated the probable presence of an effective healing principle in the gel, as an increased rate of healing was observed in about 50% of the treated rats. They also reported excellent results with the use of the rind of the leaf. These findings were considered promising, though by no means conclusive.

The investigation was therefore continued with the object in mind to definitely establish the value of aloe leaf in the treatment of X-ray burns, and if possible to isolate the healing principle if such an agent were present.

EXPERIMENTAL

General Technique

Preparation of Animal.

For this work male rats weighing about 350 Gms. were selected. On beginning the experiment, each animal was first anesthetized with ether, and the hair was removed from its back over the area to be irradiated. During the procedure of removing the hair and subsequent irradiation, anesthesia was maintained throughout by the use of an ether cone.

Irradiation of Animals.

The anesthetized animal was placed on a specially constructed board provided with clamps and hemostats which functioned to hold the rat in position by its legs. To limit the area of exposure, a lead plate with an opening the size of the area to be irradiated, was placed over the entire body. The animal was then irradiated, using for this purpose 100 kilovolts, 5 milliamperes, and T. S. D. (target skin distance) of either 8 or 10 cms. The rays were generated by a "Metalix" X-ray tube with an output of 128 r per minute in air at 30 centimeters. The features of this tube included self-protecting property, the main body being a chromium iron cylinder to which the glass was directly sealed; a diaphragm interposed between the filament and the anode to limit the "space charge" effect; and an anode designed for "line focussing" of electrons.

Aside from the inherent filter of the "Metalix" tube, none other was provided. The exposed area was limited to 16 square centimeters and 4 square centimeters, and the dosage varied from 2000 r for some to 4000 r in air for other experiments. Radiation was confined to not more than ten to twelve rats at a time.

Treatment of Animals.

The rats were kept in individual cages where they were adequately provided with food and water. The ration remained unchanged throughout the experiment. Following irradiation, the rats were divided into two groups, control and treated animals. This was done in order to overcome animal variation due to seasonal change as the experiments extended over a year's time. Only those animals which showed a fairly typical X-ray burn were used in the experiment.

It usually required 20 to 25 days for the typical X-ray reaction to develop. Treatment was therefore instituted on about the 25th day following irradiation. Control rats as well as treated ones were kept bandaged in order to approximate conditions for the two groups of animals. Only in the experiment with the volatile oil from aloe, were the control rats allowed to remain unbandaged.

The bandage consisted of Chiropodists' adhesive plaster which was cut out in the shape of a square and fixed to the rat's back around the burn area. A strip of three inch adhesive plaster was then applied

around the rat's body, with that portion which covered the burn cut out. Dressings were applied twice daily extending generally over a period of six weeks. In order to prevent the rat from tearing away its bandage, a small leather collar with tacks protruding from it was fastened around the neck. The dressing of the animals was greatly facilitated by the use of a box-like arrangement, first used and described by Rowe(loc. cit.) in his investigations on Aloe.

Materials Used in Treatment.

The materials used in treating the X-ray burns consisted of several products of the aloe leaf, including the gel, rind, latex, and volatile oil. The leaves were obtained from two different geographical sources, Florida and Porto Rico. The leaves from the both sources conformed to the description described under Aloe vera L.**. However, according to Berger(10) there are three varieties of this plant, and it was not possible to make this differentiation on the basis of leaves alone. The leaves were kept fresh by storing in a cool room.

The gel was removed after splitting the leaf open. During the course

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*We are very grateful to the following persons for having supplied us with the materials used in this investigation: Mr. T. J. Fleming, Pacific Products Co., Hawaii; Dr. Claud C. Horn, United States Horticulturist; Dr. Conrado Arsenjo, Tropical School of Medicine, Porto Rico.

**We are indebted to Prof. Fassett of the Department of Botany, University of Wisconsin, for identification of the botannical material.

of the experiment, but one leaf was used at a time and the gel obtained from it was kept in a refrigerator.

The rind when used for treatment was finely grated and then further comminuted as finely as possible. It was stored in a refrigerator when not in use.

The latex employed represented the "aloe juice" obtained from aloe plants in the production of medicinal aloe. The source of this product was Hawaii. It waspreserved in transit by the addition of chloroform, and until ready for use was kept in a cold room at 8° with a layer of toluene added. When used for treatment, it was applied in the form of a water soluble ointment• which contained 70% latex. A 3% methyl cellulose suspension in water was used for application to the controls.

The volatile oil was obtained from the distillation of aloe juice. It was applied in the form of 3% ointments•• in an aquafor base. Aquafor was also applied to the control rats.

Determination of Results.

All experiments were based upon the comparison of treated animals with the controls. Except for one phase of this investigation, treatment

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•In preparing the ointment, the toluene was first removed from the preserved latex, vacuum distilled, and the residue then redissolved in the latex. The chloroform which had also been added as a preservative was not separable but remained in solution. The ointment was composed of methyl cellulose 3%, water 27% and latex 70%, and was passed through a hand homogenizer.

••The preparation of the volatile oil was furnished by the Pacific Products Co.

with the volatile oil, all rats were carried through until final healing when the experiment was terminated. The time necessary for complete healing was regarded therefore as the criterion for ascertaining an increased rate of healing. The accuracy of this procedure was not without fault. With an irradiated area of 16 square centimeters the error involved is ±1 week; with the smaller area the error is proportionately less.

TABLE I

Treatment with Aloe Gel

Rat No.	Area of burn in sq. cm.	Weeks required for complete healing
86	6.3	28
86	7.2	22
87	7.0	15
88	8.8	25
85	8.4	24
80	6.8	15
81	7.2	17
82	6.8	16
84	6.4	8
101	6.6	18
102	7.5	20
104	10.2	16
106	6.0	13
108	6.0	13

Rats in this group received 2500 r with an irradiation area of 16 sq. cm. The gel preparation was applied twice daily for a period of six weeks. The average burnt area which developed was 7.3 sq. cm., and the average time for complete healing was 16 weeks.

TABLE II

Treatment with Rind

Rat No.	Area of burn in sq. cm.	Weeks required for complete healing
66	6.3	24
67	6.3	19
68	6.8	26
69	7.2	24

Rats in this group received 2500 r with an irradiation area of 16 sq. cm. The rind preparation was applied twice daily for a period of six weeks. The average burnterea was 6.7 sq. cm., and the average time for complete healing was 23 weeks.

TABLE XIX

Control Rats--in Relation to Tables I and II

Rat No.	Area of burn in sq. cm.	Weeks required for complete healing
60	8.7	15
61	6.9	8
62	7.2	18
63	9.0	16
64	8.4	11
73	6.7	19
74	5.3	5
75	7.3	8
76	7.5	19
77	6.8	10
85	8.4	22
87	5.4	13
88	6.3	8
89	7.2	8
107	5.9	6

These control rats received 2500 r with an irradiation area of 16 sq. cm. All rats were kept bandaged. The average burn area was 7.3 sq. cm., and the average time for complete healing was 12 weeks.

TABLE IV

Experiment with Aloe Juice--Treated Rats

Rat No.	Area of burn in sq. cm.	Weeks required for complete healing
35	9.5	17
36	9.6	7
37	8.4	16
38	9.8	18
39	7.5	20

Experiment with Aloe Juice--Control Rats

Rat No.	Area of burn in sq. cm.	Weeks required for complete healing
40	10.9	15
41	8.1	9
42	8.4	9
43	9.0	8
44	7.2	7

Each group of rats received 2000 r individually with an irradiation area of 16 sq. cm. All rats were kept bandaged. Average burn which developed in treated rats was 8.8 sq. cm., and average time required for complete healing was 16 weeks. Treatment with the aloe juice preparation continued for 6 weeks, twice daily. Average burn area for controls was 8.8 sq. cm., and average time required for complete healing was 10 weeks.

TABLE V

Experiment with Volatile Oil

TREATED RATS		CONTROL RATS	
Rat No.	Area of burn in sq. cms.	Rat No.	Area of burn in sq. cms.
27	11.2	9	12.2
28	13.3	10	12.2
29	12.6	15	10.5
30	12.2	16	10.9
31	11.9	17	9.6
32	12.3	18	10.5
33	11.9	19	12.8
		25	14.0
		34	11.7

These rats were given individually 3600 r, involving an irradiation area of 16 sq. cms. The untreated rats were left unbandaged. Average burn area which developed in treated rats was 12.2 sq. cms., in control rats 11.8 sq. cms. Rats in the treated group received one daily application of the volatile oil preparation for the first two days and two daily applications thereafter for the next 14 days. At the end of the 16 day treatment period, the treated rats appeared in a much worse condition. The burn areas in both groups of rats became too irregular to measure with any accuracy following the period of treatment.

TABLE VI

Experiment on Comparative Healing

Rat No.	Area showing greater healing end of 5 weeks.	Days required for complete healing. Ant.	Post.
92	Post.	55	51
93	Equal	58	56
95	Ant.	52	55
96	Ant.	48	49
97	Ant.	36	42
111	Ant.	34	43
112	Ant.	37	43
113	Ant.	50	62
114	Ant.	28	38
115	Ant.	36	61
116	Ant.	33	38
117	Ant.	38	42
118	Equal	41	40
119	Post.	40	37
120	Ant.	50	47
121	Ant.	34	37
122	Equal	40	39

Each of the rats in this group was subjected to irradiation over two exposed areas on the back of the animal, approximately 1 to 2 inches

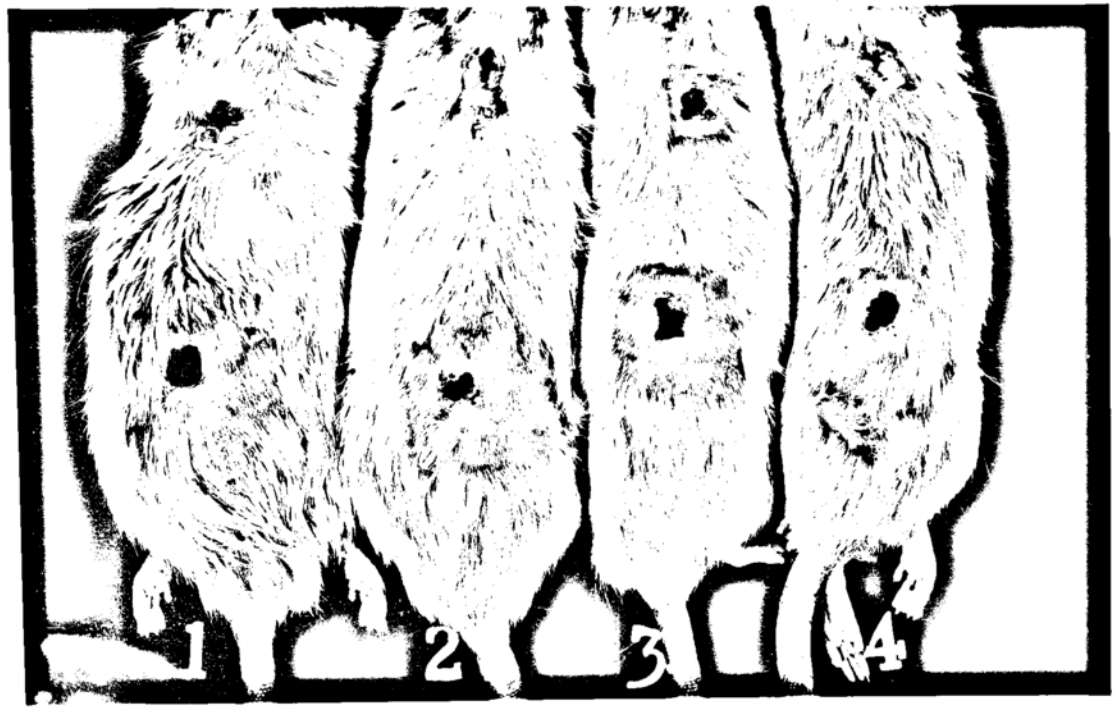

Fig. I. Rats No. 111, 112, 113, and 114, showing greater healing of anterior areas compared to posterior ones. Each area received 4000 r, involving an irradiation area of 4 sq. cm. No treatmentof any sort was applied. Photograph was taken 31 days following irradiation.

apart. Each area received 4000 r and was limited to 4 sq. cm. The rats were kept unbandaged, and without application of any treatment. In 70% of the animals, the anterior burn was found to have healed faster than the posterior burn when compared at the end of 5 weeks following irradiation. Both burns appeared the same in 18% of the animals, with the posterior area most improved in only 12% of the cases.

DISCUSSION

In view of the favorable reports on the use of Aloe vera leaf in X-ray burn therapy, the primary objectives undertaken in this investigation were first to find a preparation which was therapeutically active and then to attempt isolation of the effective principle. While treatments generally had been carried out by application of the gel to the burn, Rowe et al. considered that the healing agent may possibly be concentrated in greater amounts or in a more effective form in the rind of the leaf. The aloe leaf was therefore resolved into its component parts--rind, gel, latex and also a volatile oil. Each of these products was tested in order to determine which would be most suitable and most effective as starting material for isolation of the active principle.

The procedure finally adopted for studying its healing effect of aloe was different from that employed in the earlier studies. In the previous work rats were irradiated with two different areas of the animal's back exposed, one anterior and the other posterior. It was necessary to limit the area of these burned parts in view of the relatively small size of the animal. Under these circumstances, spontaneous healing was found too rapid for a proper study of comparative healing. A much more important criticism of this procedure was the disadvantage of comparing areas ofdifferent parts of the animal's body. It would perhaps be expected that a lesion on one part of the body may heal much more rapidly than a

similar lesion located elsewhere on the body. These differences could be expected, especially on account of variable thickness of skin and dissimilar vascularity. On these considerations, the method for studying the rate of healing was therefore modified. A single, much larger X-ray burn was developed on the back of the rats; half of these animals then served as controls, while the others underwent treatment with one of the aloe preparations.

The application of aloe gel led to results which were entirely negative, for an increased rate of healing was definitely not observed. As a matter of fact the rats treated with this product required a greater length of time for complete healing when compared to the controls; whereas the treated areas required an average of 16 weeks, those used as controls required an average of 12 weeks. It was therefore concluded that this preparation was therapeutically ineffective in the treatment of these burns. It was considered highly improbable that the preparation lost any of its effectiveness from inactivation for the aloe leaves were all in good condition. Furthermore, care was taken to use but one leaf at a time and to store the gel when not in use in a refrigerator.

Negative results were also experienced with the use of the rind. Although only a limited number of rats were treated, healing was so adversely affected that there could be no question that the rind preparation used was devoid of any effective healing principle. Whereas the treated

rats required an average of 23 weeks for complete healing, the control rats required an average of 12 weeks.

Treatment with the latex preparation resulted in a much aggravated condition of the burn which was believed to be due to the presence of a large amount of resin in the latex. The average time required for complete healing of the treated areas was 16 weeks as compared to an average of 10 weeks for the untreated areas. A similar observation was made by Rowe who found that aloe (dried latex) affected adversely normal healing.

As none of the component parts of the Aloe leaf proved therapeutically active, one possibility was considered. It is known that a trace of volatile oil is present in the latex of Aloe leaves. The volatile oil was therefore tested out, but it also appeared to be without beneficial effect on the healing of X-ray burns.

As the earlier results obtained by Rowe could not be duplicated, it was considered possible that an explanation which resolved on the basis of the two different experimental procedures employed could be found. The previous method involved the comparison of two burn areas located on different parts of the animal body. It could therefore be expected that healing of each area may not proceed to the same degree and extent within a given period of time. It was actually observed that in 70% of the rats the anterior burn would heal naturally faster as compared to the posterior burn at the end of 8 weeks. This was the basis of Rowe's method for ascertaining an increased rate of healing.

It was also observed that the anterior area was first to develop its burn, and in this respect the anterior area was less resistant to irradiation. In addition, the difference in rate of healing was found proportionately greater the further apart were these burns on the rat's back. This is just as would be anticipated.

Another criticism which may be directed against the earlier method, was the practice of allowing the untreated area to remain unprotected, whereas the treated area was kept bandaged. This would be a very important factor in effecting the results of an experiment. In view of these findings it is doubtful whether an increased rate of healing was actually observed in the earlier studies on Aloe.

SUMMARY

In comparatively recent times, aloe gel was introduced for the treatment of X-ray burns with limited evidence of any medicinal value.

Promising results were first reported from experiments on rats, used for treatment and the controls. However, the experimental method might be considered faulty; for the treated anterior area tended to heal normally faster compared to the untreated posterior area. A very important criticism of the method was also the practice of allowing the untreated area to remain unbandaged and exposed to irritation.

The experimental method employed limited treated and untreated X-ray burns, developed on rats, to similar conditions, as far as was possible. Negative results were experienced with aloe gel, rind, latex, and the volatile oil. It was concluded that aloe gel as well as the other products tested were of doubtful value in the treatment of X-ray burns.

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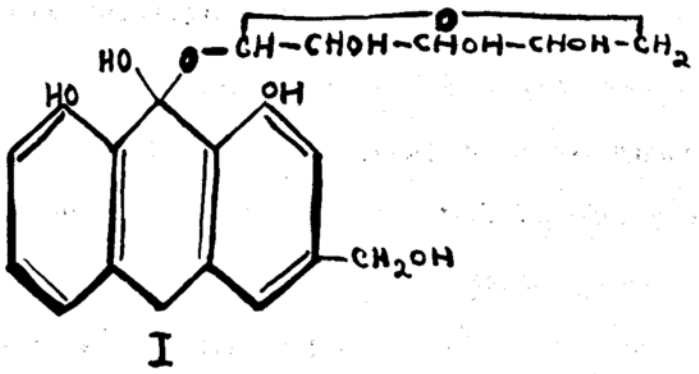
COMPARATIVE CATHARTIC ACTION OF ALOIN, ALOE-EMODIN, AND ALOE-EMODIN ANTHRANOL.

INTRODUCTION

The drug, aloes, has been employed in medicine as a cathartic for many centuries. The early Greeks and Arabians were apparently familiar with its use as the drug was frequently referred to in the writings of Pliny, Dioscorides, and Celsus.

Despite the continued use of aloes throughout the centuries, it was not until 1851 when the Messrs. T. and H. Smith(1) succeeded in isolating a substance which could be regarded as the effective therapeutic principle of the drug. The isolated product was called "aloin." Subsequent pharmacological studies(2) have demonstrated that aloin affects mainly the large intestine of the intact animal, and that 8 to 12 hours and even longer are required for it to act. It has been shown to be without action on isolated intestinal muscle. From the work of Leger(3) it appeared that aloin was a glycoside composed of a aloe-emodin and d-arabinose. It was therefore anticipated that the long delay in action of aloin was dependent upon the hydrolysis of the pentoside with the liberation of aloe-emodin. More recent work on the chemistry of aloin has led to conflicting views in regard to its structure. Both Hauser(4) and Rosenthaler(5) advanced pentoside structures which involve aloe-emodin anthranol rather than aloe-emodin. Whereas Simonsen(6) regards aloin not as a glycoside at all but as a complex structure which is best designated by a C_{16} formula instead of the C_{20} formulae which the pentoside compounds require. However, aloin may

be regarded as an arabinoside of the hydrate of aloe-emodin anthrone, I. The latter compound would easily revert to the anthranol. The fact is



I

that aloin is hydrolyzed with considerable difficulty; furthermore, enzymes are without effect on the hydrolysis of this unusual compound.

Studies on synthetic anthraquinone derivatives(7) as cathartics have been performed, though these compounds do not appear to be as suitable as are the natural products. Indeed, no practical application of any significance has been realized of these synthetic products.

This investigation was undertaken in an attempt to establish which of the degradation products of aloin is to be regarded as the agent directly responsible for the therapeutic action of aloin. At the same time it offered an opportunity to compare pharmacologically anthraquinone and anthranol compounds. So far as is known, such a comparison has never been accomplished; in fact it has never been shown experimentally that anthranols may possess cathartic action.

Preparation of Aloe-Emodin.

Aloe-emodin was prepared from aloin by the method of Simonsen(6). In this procedure 10 Gm. of pure aloin and 50 Gm. of ferric chloride were heated with 200 cc. of water at a temperature of 125°C. for 4 hours. The mixture was then cooled, and the precipitate which formed was removed, washed, and dried. It was then extracted with toluene from which aloe-emodin was allowed to crystallize.

A portion of the product was converted to the monosodium salt by adding an equivalent amount of alcoholic sodium hydroxide and evaporating to dryness.

Preparation of Aloe-Emodin Anthranol.

Aloe-emodin anthranol was prepared from aloin by the method of Houser(4). Borax, 10 Gm., and aloin, 10 Gm. were dissolved in 100 cc. of water and heated for one hour on a water bath. The solution was acidulated with hydrochloric acid and the precipitate which formed was collected, washed, and dried. It was finally extracted with benzene from which aloe-emodin anthranol crystallized.

The monosodium compound was prepared by the addition of theequivalent amount of alcoholic sodium hydroxide, followed by evaporation to dryness.

Studies on Isolated Intestinal Muscle.

A strip of intestinal muscle was prepared from a rabbit and suspended in Locke's solution, kept oxygenated by bubbling air through it. A temperature of 38° was maintained. The normal muscular contractions were recorded on a kymograph. Aloe-emodin and later aloe-emodin anthranol, each dissolved in water as the monosodium salt, were added to the bath.

Studies on Rats.

For this part of the experiment male rats weighing 300 Gm. were selected and kept on a prepared diet (Frishies). The ration and water were supplied ad libitum.

The determination of the minimum effective dose was performed on a group of 10 rats which were not again employed for testing another cathartic dose until they became restored to normalcy. Generally, an interval of about 5 or more days was allowed to elapse. Prior to the administration of a cathartic the animals were not given any food for three hours. The laxative agent was then given mixed with a small quantity of comminuted food, which was placed in a feeding cup firmly attached to the cage in order to prevent tipping. By such an arrangement the animals consumed the food mixture within 15 minutes. A cathartic effect was indicated by a change in the character of the stools. The feces became semi-solid in contrast to the normally solid and well formed stools.

The onset of action was determined after giving to rats 20 mg. of

each product. From such a drastic dose, the first signs of catharsis were easily recognized. The results were duplicated.

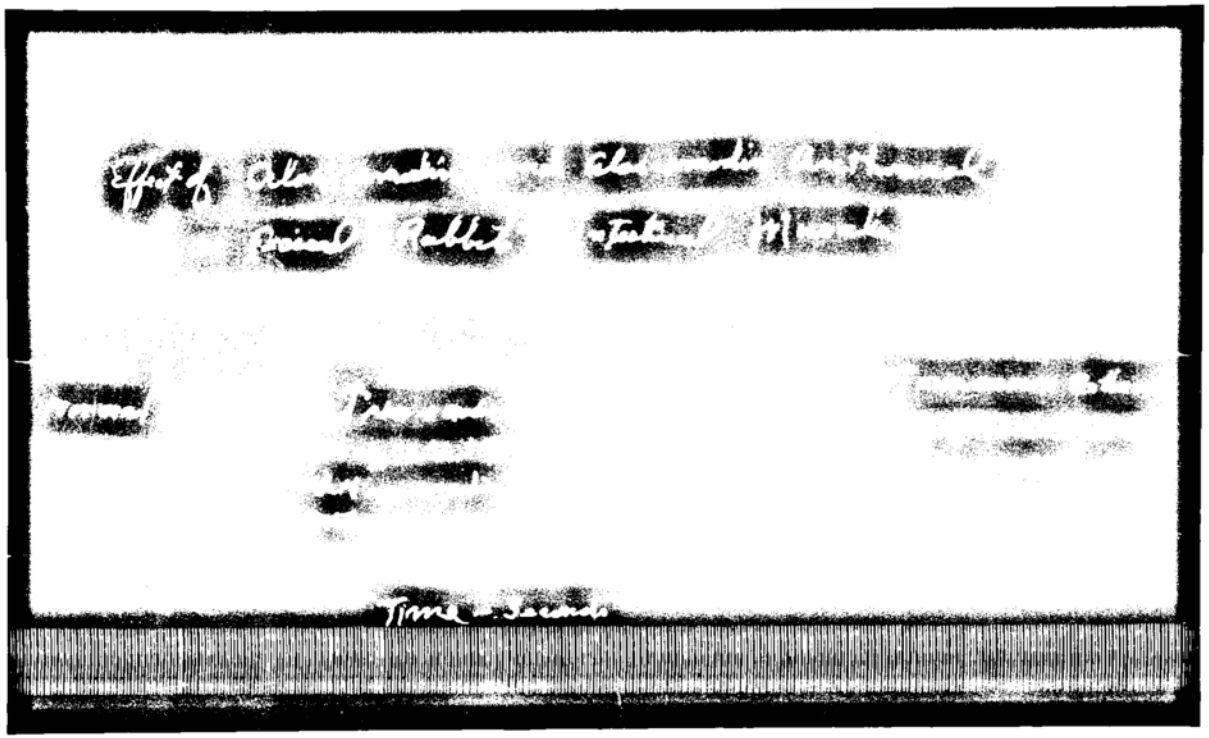


Fig. I.

(See pages 37 and 41)

TABLE II

Comparative Action of Aloin, Aloe-Emodin, and Aloe-Emodin Anthranol.

Product	Minimum Effective Dose Percentage of Rats Showing Effect			Time for Effect to First Appear
	2 mg.	5 mg.	10 mg.	
Aloin	0%	80%	100%	12-13 hours
Aloe-Emodin	0%	20%	80%	8 hours
Aloe-Emodin Anthranol	0%	40%	70%	4-4½ hours

RESULTS

In view of the fact that aloin was previously shown to exert no immediate direct action on isolated intestinal muscle, it appeared worthwhile to conduct a similar experiment with aloe-emodin and aloe-emodin anthranol. These two latter compounds were tested out as their sodium salts on isolated rabbit's intestinal muscle and were found to be without immediate direct action (see kymograph record). The comparison of the cathartic action of these compounds was therefore continued with the use of intact animals.

Of the three products examined for their cathartic effects on rats, aloin was found to be the most active on the basis of minimum dose; whereas aloe-emodin and aloe-emodin anthranol appeared to be of the same order of activity. Although these comparisons were conducted on a weight basis rather than on the molecular relationship of these compounds, the results are still significant. On a weight basis aloin is about 1.4 times as active compared to the other substances, and by a calculation on a molecular basis* it would be about 2 times as active.

In these experiments aloin required 12 to 13 hours to act, whereas aloe-emodin required 8 hours and aloe-emodin anthranol about 4 hours.

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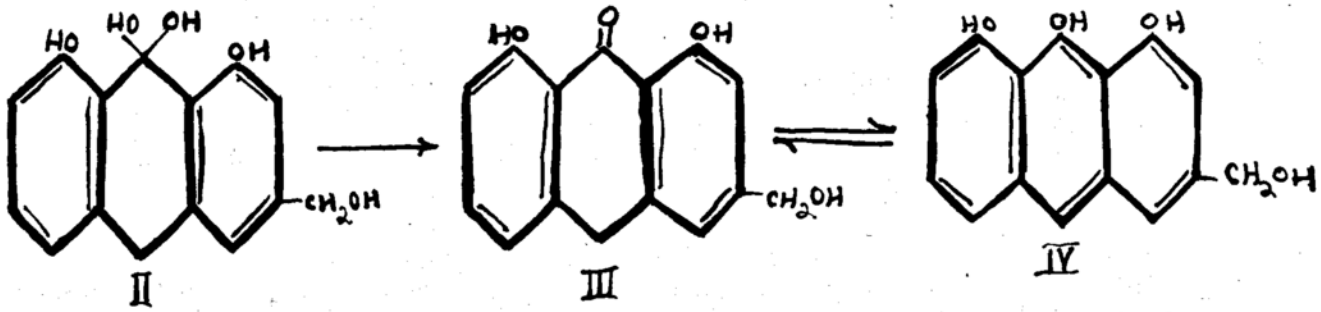
*Molecular weights: aloin, 404; aloe-emodin, 270; aloe-emodin anthranol, 256; the difference in molecular weights of the latter two compounds too small to consider.

DISCUSSION

As aloin is truly a glycoside, its long delay in action can be attributed to the slow rate of hydrolysis and liberation of the aglycone. The latter agent, II, would be expected to be very unstable and be transformed to the anthrone, III. In solution the anthrone would exist in equilibrium with the isomeric anthranol, IV, the relative proportion of each depending upon the nature of the solution. For this reason, the



experiment was limited to the study of one of these isomeric forms, aloe-emodin anthranol. By the administration of aloe-emodin anthranol the time required for inducing catharsis was reduced from 12 or 13 hours to about 4 hours.

One observation was made, which requires an explanation; for it was noted that aloin considered either on a weight or molecular basis was more active than aloe-emodin anthranol. This observation also applied to aloe-emodin. A plausible explanation may be advanced on the basis of solubility as these hydroxy anthracene derivatives are extremely insoluble com-

pounds.

Although the intestines have an alkaline reaction the pH is insufficient to account for the solubility of these phenolic compounds, which are insoluble in sodium bicarbonate solution, but soluble in sodium carbonate solution. The alkalinity of the intestines is therefore not a major factor to be considered.

Hydrolysis of aloin, a water soluble compound, would lead to the liberation of the anthrone or anthranol in a molecular condition which would favor, due to the greater surface area, the rate of solution as well as the quantity in solution in a given time. A close analogy is encountered with the simple, inorganic, laxative agent, calomel. From common experience, it is known that trituration of mercurous chloride, results after taking, in a greater purgative action. Apparently, the pharmacological action is dependent upon particle size which in turn influences solubility.

Text books on pharmacology frequently state that the action of aloin is increased by giving iron salts and alkalies. The action of these agents is now understandable. Ferric salts would be expected to oxidize aloin which ordinarily hydrolyzes very slowly to aloe-emodin, and alkalies would increase the rate of hydrolysis of aloin and possibly have some effect upon the solubility of the aglycone.

In contrast to the many anthraquinone derivatives which have been investigated, the anthranols have never been thought of as cathartics.

Although, a few medicinal plants, Rhamnus Cathartica L. and R. Frangula L., are known to contain, in addition to the many anthraquinone compounds present, anthranol glycosides. Indeed, the writer's observation of the cathartic action of aloe-emodin anthranol marks the first indication that anthranols in general may possess a cathartic action. Although but a single anthranol was tested, there is no reason for regarding aloe-emodin anthranol as an exception to the general class of anthranols.

It is hoped that further study of anthranol derivatives will lead to a worthwhile development in the field of laxation. There is at least an indication that anthranols may be superior to anthraquinones as laxatives for aloe-emodin anthranol required less time in which to act.

SUMMARY

Aloe-emodin and aloe-emodin anthranol were tested on isolated rabbit's intestinal muscle and found to be without immediate direct action.

Aloin required about 12 hours to induce catharsis compared to 8 hours for aloe-emodin and 4 hours for aloe-emodin anthranol.

On a molecular or weight basis, aloin was more effective than aloe-emodin or aloe-emodin anthranol. An explanation was advanced on the basis of solubility.

The observation of the cathartic action of aloe-emodin anthranol was regarded as of fundamental importance for it may lead to the synthesis of new laxative agents.

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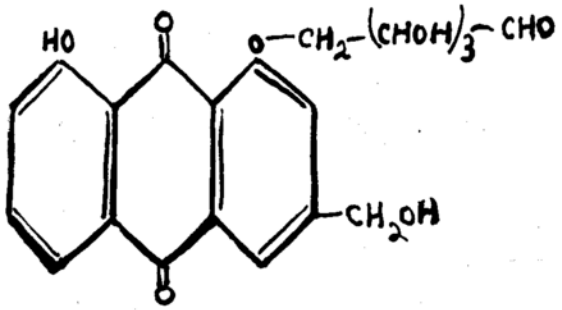
CONSTITUTION OF ALOIS

INTRODUCTION

For over half a century the purgative principle, aloin, has been the subject of numerous investigations. As a result there has accumulated over these many years an extensive literature concerning its constitution. While it is generally assumed that this principle is a penteside, this view has been strongly challenged within the last decade. Indeed, it has become the subject of a remarkable controversy. The fact remains that the chemistry of aloin is imperfectly understood, and its constitution is therefore unknown.

On the sole basis that aloin (barbaloin) yielded on decomposition both aloe-emodin and d-arabinose, Leger(1) proposed the following structure:

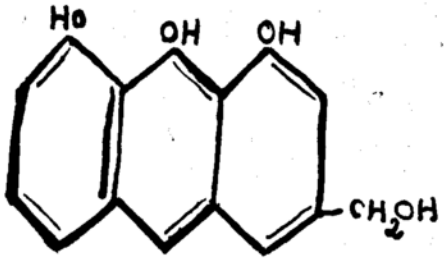


This requires the formula, $C_{20}H_{18}O_9$. The aglycone, aloe-emodin, was characterized by Oesterle(2) as 1,8-dihydroxy anthraquinone carbinol-3, which other investigators(3) confirmed.

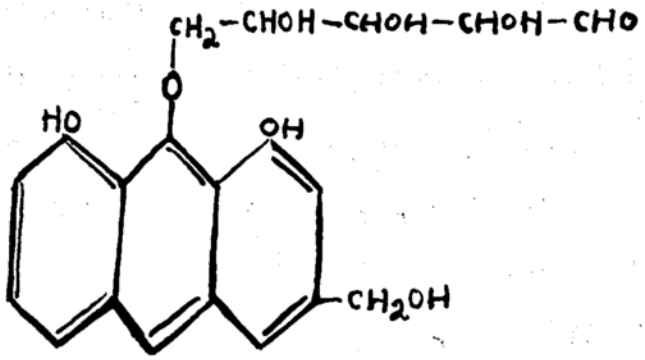
Unlike other glycosides aloin does not hydrolyze easily. Drastic means, such as the use of sodium peroxide, are required to bring about hydrolytic decomposition. Furthermore, enzymes do not seem to act upon it. On these accounts, Leger believed the sugar was attached to aloe-emodin through its end primary hydroxyl forming an ether; in this manner it differed from the usual glycosidal linkage. Reduction of Fehling's solution by aloin and the formation of a penta-acetyl derivative on this formula supported such a structure.

Some inaccuracies in the Leger formula were pointed out by Rosenthaler(4). A compound according to such a formulation would be expected to form an osazone or a hydrazone, but none could be obtained either with phenyl-hydrazine or with p-nitro-phenylhydrazine. In addition, the terminal CHO group should easily undergo upon mild oxidation, transformation to an acid of the same number of carbon atoms. Such a C_{20} acid likewise could not be isolated by Rosenthaler.

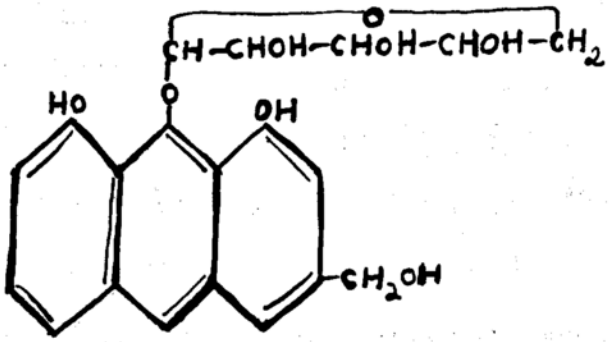
Hauser's work(5) shed additional light on the decomposition of aloin. The degradation of aloin by means of borax (Schunteton reaction) was found to yield an anthranol rather than an anthraquinone. One of the structures advanced for the representation of this anthranol was later verified by McDermol and Gardner(6).



The anthranol obtained can be converted to aloe-emodin through aerial oxidation in alkaline solution, a reaction which explains favorably the formation of aloe-emodin in some aloin decompositions. Hauser therefore suggested an aloe-emodin anthranol d-arabinoside with an ether linkage for aloin. This structure, however, bears the same disadvantage as the Leger formulation. In addition, the formula $C_{20}H_{20}O_8$ which is required does not agree with the analytical evidence found for aloin.



Based on the consideration that boron effects a fission of aloin into aloe-emodin anthranol and d-arabinose, Rosenthaler(7) advanced a simple penteside structure. The compound has the empirical formula $C_{20}H_{20}O_8$ also and the structure,



Rosenthaler's formulation does not take into consideration the difficulty with which aloin is hydrolysed, together with the small yield of arabinose. At least Rosenthaler has not been able to advance a satisfactory explanation for these anomalies. Goldner(8) applied Kreber's method for estimating the extent of hydrolysis of aloin. With the use of sodium perborate, 30% hydrolysis of aloin was indicated, while sodium peroxide resulted in 8%. Hydrolysis with acids and bases gave much lower values.

Another argument against acceptance of such a structure was the failure by Simonsen(9) to isolate any sugar in the Schmiedeberg reaction.

Foster and Gardner(10) prepared -d-glucosides of 1,8- and 1,8-dihydroxyanthraquinones and of 1,8-dihydroxy 3-methylanthraquinone and studied the hydrolytic rates of these substances in diluted hydrochloric

acid, potassium hydroxide) and borax. Their results indicated rapid hydrolysis of these synthetic glucosides in great contrast to aloin. However, as aloin is to be regarded as an anthranol derivative rather than as an anthraquinone, no conclusion can be drawn from Foster and Gardner's study.

While the formulas advanced by Léger, Hauser, and Rosenthaler all depend upon a C_{20} compound, Simonsen(loc.cit.) introduced some evidence for a C_{16} compound, a formula which was first advocated by Tilden(11). Ordinary chemical analysis does not readily distinguish between C_{16} and Leger's C_{20} formulas. In contrast, the analysis of aloin eliminates both Rosenthaler's and Hauser's formulas.

TABLE I

Formula		% C	% H
$C_{16}H_{18}O_7$	(Tilden)	59.63	5.59
$C_{20}H_{18}O_9$	(Léger)	59.70	4.48
$C_{20}H_{20}O_8$	(Hauser, Rosenthaler)	61.86	5.15
Found*			
Jowett and Potter		59.6	5.6
Léger		59.90	5.37
Greenwald		59.86	5.42
Tschirch and Hoffmeyer		60.08	5.44
Rosenthaler		58.37	5.41
Schmidt		59.28	5.61

*Represents average of several analyses each.

It will be noted that the values found for hydrogen are in greater agreement with a C_{16} compound. Indeed, the work of earlier investigators would seem to confirm the C_{16} formula: Schmidt(12) $C_{16}H_{18}O_7$; Tremann(13) $C_{42}H_{60}O_{21}$; Greenwald(14) $C_{16}H_{18}O_7$; Tschirch and Pedersen(15) $C_{16}H_{18}O_7$; Tschirch and Hoffmeier(16) $C_{16}H_{18}O_7$; Aschman(17) $C_{16}H_{18}O_7$; Leger(18) $C_{16}H_{18}O_7$; Jowett and Potter(19) $C_{16}H_{18}O_7$.

Molecular weight determinations (9,17,19,20) which would easily solve the problem, result in a variety of values, apparently on account of the abnormal behavior of aloin in solutions. Consequently, such determinations cannot be applied for the support of any formula.

The strongest evidences for the support of the C_{16} formula are certain halogen derivatives. Thus with bromine water, aloin is converted to a tri- or tetra-bromine derivative according to which formula is applied.

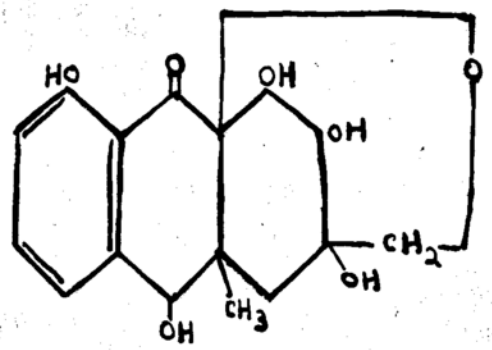
TABLE II

Formula	% Br	% C	% H
$C_{16}H_{15}O_7Br_3$ (Simonsen)	42.9	34.3	2.7
$C_{20}H_{14}O_9Br_4$ (Leger)	44.6	33.4	1.9
$C_{20}H_{16}O_9Br_4$ (Hauser, Rosenthaler)	43.8	34.1	2.3
Found:			
Leger	42.6	34.1	2.7
Gibson and Simonsen	42.8	....	...
Rosenthaler	42.0	....	...
Greenwald	43.2	34.5	2.8

*Average of several determinations.

The analysis of the chlorine derivative of barbaloin is equally convincing in the support of $C_{16}H_{18}O_7$. While several investigators concede that these halogen compounds are best represented by the C_{16} formula, they are skeptical about accepting these as true derivatives of the parent substance aloin. Hence one of our problems was to demonstrate the halogen compounds as constituting true derivatives.

Considering aloin as $C_{16}H_{18}O_7$, involving potential aloe-emodin and arabinose units, Simonsen proposed the following structure, with the disposition of one or two hydroxyls debatable:

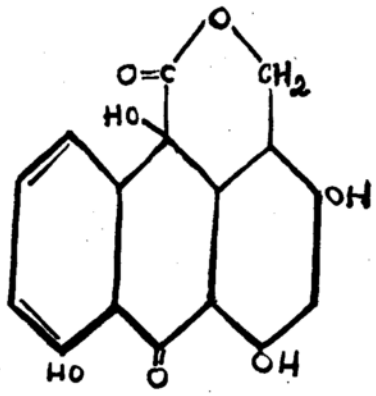


In upholding this formula and in general a C_{16} compound, Simonsen pointed out to a 70% yield of aloe-emodin obtained by oxidation of aloin with $FeCl_3$, in contrast to a 68.9% theoretical yield when based on the Leger formula. In addition to this, a colorless pentamethyl ether was obtained by the action of silver oxide and methyl iodide, whereas a colored compound would be expected on the presumption of other formulas advanced.

However, these observations have been criticised on the grounds that the yield of aloe-emodin may not have been entirely pure; and the use of silver oxide might possibly effect a change in aloin as is similarly brought about by Fehling's solution.

Apparent obstacles to the acceptance of this structure are the presence of a ketone group and the difficulty involved in the degradation to arabinose. The latter reaction would involve rupture of three C-C bonds, a course which is hardly conceivable. However, this may not be considered a valid objection if we consider how small the yield of arabinose actually is. The structure also does not readily allow transformation to aloe-emodin anthranol, or conversion to anthraquinone derivatives which are easily produced from the action of oxidising agents.

A different structure, but based on a C_{16} formula, was previously advanced by Robinson and Simonsen(21), though on inconclusive grounds. It is as follows:



Although it would appear that the C_{16} formula is proved by the analyses of aloin as well as of bromobarbaloin, prepared by bromination in aqueous solution, other derivatives have been obtained which refute a C_{16} formula. Leger obtained a different halogen compound by bromination of aloin in hydrobromic acid solution. The analysis of this compound supports somewhat Leger's own formula; it would seem to disprove the C_{16} formula, and is less convincing for Hauser's or Rosenthaler's formula.

TABLE XXI

Formula		% C	% H	% Br
$C_{20}H_{15}O_9Br_3$	(Leger)	37.5	2.7	37.5
$C_{20}H_{17}O_8Br_3$	(Hauser, Rosenthaler)	38.4	2.7	38.4
$C_{16}H_{16}O_7Br_2$	(Simonsen)	40.0	3.3	33.3
Found				
Leger	I	38.44	3.37	36.67
	II	37.89	3.42	36.65
Simonsen	I	37.7	3.0	37.6; 37.8

The acetyl derivative of Leger's tribromo-barbaloin was prepared by Simonsen(22) and found to agree with the formula, $C_{20}H_{15}O_9Br_3$. However, the analysis of the methyl-ethers failed to agree with the formula, unless

it is assumed that one methyl radicle was introduced during the process of methylation.

It is apparent that the reports on the chemistry of aloin have resulted in a very confusing situation which is indeed most perplexing. Not one of the formulas advanced is in general agreement with the analyses of the many derivatives of aloin. In view of these conflicting evidences, neither of the formulas can be regarded as correct. It was therefore necessary to reinterpret the findings already obtained from the numerous investigations on aloin, in order to decide upon the correct molecular formula. Supported by additional chemical studies it was possible to derive a logical structure for the representation of aloin.

EXPERIMENTAL

This chemical investigation was made especially difficult on account of the physical properties of the aloin derivatives. These products were generally non-crystalline, and failed to give sharp melting points. Attempted purification through crystallization frequently failed to yield pure compounds.

The aloin used in this work was purified by many crystallizations from various solvents, water, methyl alcohol, chloroform, ethyl acetate, acetone, or mixtures of these. The aloin purified in this manner was composed of friable, lustrous crystals, which became easily reduced to a light yellow powder. The melting point of the anhydrous material was 147°C. The purified aloin did not give the Klunge reaction(23), whereas impure aloin responded to the test readily. This indicated the absence of iso-barbaloin as the Klunge reaction, according to Leger, is specific for iso-barbaloin rather than for barbaloin.

Analysis of Aloin

A carbon and hydrogen determination was performed upon a highly crystalline sample of aloin, last crystallized from ethyl alcohol. It was dried in a vacuum over P_2O_5 at a temperature of 110°C. for 8 hours.

Found: I, 58.60% C; 5.70% H; II, 58.36% C; 5.62% H;

Calculated for $C_{20}H_{22}O_9$: 59.11% C; 5.42% H.

Test for Pentose

Molisch Test.

The Molisch test was applied to an aqueous solution of aloin. The test repeatedly gave negative results.

Conversion to Furfural.

A sample of aloin was distilled with 12% hydrochloric acid. The distillate gave a pink color with aniline acetate.

The above hydrochloric acid solution of aloin was refluxed for two hours and then distilled again. Phloroglucinol in hydrochloric acid solution was added to the distillate with the formation of the typical furfural-phloroglucinide condensation product.

Test for Carbonyl Group

The following tests were applied to aloin in aqueous solution: Fehling's test--positive; Tollens' test--positive; Schiff's test--negative; oxime formation--negative; semicarbazone formation--negative; phenylhydrazone formation--negative.

Test for Phenol

Reaction with Ferric Chloride.

An aqueous solution of aloin gave with ferric chloride a brown color which did not disappear on heating.

Bromination.

An aqueous solution of aloin was readily brominated upon the addition of bromine water.

Solubility in Alkali.

Aloin was found to be extremely soluble in a diluted solution of sodium hydroxide. Tetrabromo-barbaloin, which is a very insoluble compound was likewise found to be soluble in the alkali solution.

Coupling.

To a solution of diazotized sulfanilic acid prepared in the usual manner, was added a dilute solution of aloin. A red colored solution was obtained. No reaction occurred when a saturated solution of tetrabromo-barbaloin was used.

Determination of Phenolic Hydroxyls.

Anhydrous tetrabromo-barbaloin in weighed portions was treated with variable amounts of sodium hydroxide solution prepared from metalic sodium and freshly boiled distilled water. Heat was applied to the alkali solution to aid the halogen compound to dissolve. A slight excess of a solution of silver nitrate, free from carbon dioxide, was added to each of the solutions of sodium tetrabromo-barbaloinate. The color of the precipitate which formed was observed. A bright orange colored precipitate indicated the formation of silver tetrabromo-barbaloinate; whereas the production of a brown colored precipitate indicated the presence of silver oxide.

Equivalent of Alkali Added*	Color of Precipitate Produced
0.8	orange
1.8	orange
2.1	slightly brown
2.2	brown
2.3	brown

*Based on formula $C_{20}H_{18}O_9Br_4$.

As the end point occurred when about two equivalents of alkali was added, tetrabromo-barbaloin must therefore contain two phenolic groups.

Acid Hydrolysis

Hydrochloric Acid 6%.

Four grams of aloin were dissolved in 80 cc. of 6% hydrochloric acid and refluxed on a steam bath for 3½ hours. It was then filtered to remove a negligible amount of black material. The filtrate was chilled and aloin was recovered unchanged.

Hydrochloric Acid 12%.

Two grams of aloin were dissolved in 150 cc. of 12% hydrochloric acid and refluxed for four hours. The solution was then distilled until 250 cc. of distillate were obtained, replacing the distilled acid with additional 12% acid and maintaining a volume of about 100 cc. in the distillation flask.

The collected distillate was filtered and 0.76 Gm. of phloroglucinol,

diresocin free, dissolved in 12% hydrochloric acid was added. The solution was made up to 400 cc. with the acid, and the mixture was allowed to stand over night. The phlorglucinide was weighed and the pentose calculated according to the A. O. A. C. method(24). The yields were: I, 0.012 Gm.; II, 0.037 Gm.

The residue in the distillation flask was filtered, whereby a black insoluble material was obtained: I, 0.80 Gm.; II, 0.88 Gm. A negligible amount of material was extracted from it with benzene. The black product appeared to be largely carbonaceous.

The filtrate was partially neutralized with sodium hydroxide solution, acid to litmus but alkaline to Congo red, and extracted with warm benzene. A yellow product, 0.15 Gm., crystallized from the benzene solution. It gave with alkali a yellowish solution; produced with concentrated sulfuric acid a yellowish-red color; and an alcoholic solution with ferric chloride resulted in a brownish-red color which disappeared on heating. A solution of the product in alkali became deep red on passing air through it. The typical color reaction did not result with Mecke's reagent, nor was Fehling's solution reduced in the cold (differences from aloe-emodin anthranol and -anthrone). The product was oxidized by means of hydrogen peroxide in glacial acetic acid to aloe-emodin, m. p. 223°C. The latter was converted to an acetate, m. p. 175-176°C., with acetic acid anhydride and sodium acetate.

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selenous acid, 5%, in concentrated sulfuric acid.

Borax Hydrolysis

Aloin, 8 Gm., and sodium borate, 16 Gm., were dissolved in 100 cc. of water and refluxed on a steam bath for 1½ hours. It was then cooled, made acid with hydrochloric acid, and filtered.

The insoluble portion was washed, dried, and extracted with benzene to yield aloe-emodin anthranol, 0.7 Gm.

The filtrate was tested for arabinose, formaldehyde, formic acid, and methyl alcohol. All tests responded negatively. The formation of carbon dioxide or carbon monoxide in the reaction was also tested for with negative results.

Pyrolysis of Aloin

A sample of aloin was heated in a reflux apparatus to a temperature of 250°C. Any gas which formed was allowed to pass from the open end of the condenser into a solution of barium hydroxide contained in a test tube. The latter was connected in turn to a soda-lime tower, in order to prevent carbon dioxide in air from reacting with the hydroxide. The procedure, modified by first allowing the vapors to pass over heated copper oxide, was repeated. By these methods it was found that carbon monoxide and carbon dioxide were not produced on pyrogenic decomposition of aloin.

Preparation of Heptaacetyl-barbaloin

Anhydrous aloin was refluxed with a large excess of acetyl chloride on a water bath for 1 hour. The excess acid chloride was largely removed through distillation and the residue was poured onto crushed ice. The acetyl compound which at first appeared in a liquified condition was rubbed with a stirring rod until the compound became semi-solid. The supernatant liquid was removed and the derivative was allowed to stand in diluted sodium carbonate solution when solidification occurred. It was purified by solution in acetic acid followed by precipitation with water. Crystallization from solvents proved unsuccessful, although from an acetone-alcohol-water mixture a yellow powder separated out, but even this could not be repeated.

The acetylated product was found to be to a very light yellow substance, m. p. 93-95°C. It was very soluble in ether, benzene, acetone, and alcohol, slightly soluble in methyl alcohol, insoluble in water and alkali. It was brominated on standing by bromine in benzene solution.

The acetyl value of the compound was determined by saponification with 20% sodium hydroxide, followed by acidification with phosphoric acid and steam distillation. The distillate was titrated with standard sodium hydroxide and phenolphthalein as an indicator. Required: for 0.3243 Gm. of sample, 32.2 cc. 0.1 N. NaOH, equivalent to 42.8% acetyl; for 0.2704 Gm., 27.2 cc. 0.1 N. NaOH, equivalent to 43.3% acetyl; calculated for $C_{20}H_{15}O_9(CH_3CO)_7$: 43.0% acetyl.

Preparation of Hexaacetyl-barbaloin

Five grams of anhydrous aloin were dissolved in 50 cc. of acetic acid anhydride, containing a few drops of concentrated sulfuric acid, and refluxed for about one hour. The excess acid anhydride was largely removed by distillation. The residue was poured onto cracked ice and stirred until the acetate solidified, after which it was washed with sodium carbonate solution and with water. It was purified by repeated solution in glacial acetic acid and dilution with water to precipitate it. Other attempts to crystallize it were unsuccessful.

The compound was yellowish green and melted at 140-141°C. It was found to be insoluble in alkali and soluble in acids, acetone, ether, and benzene. The compound reacted negatively towards phenylhydrazine.

The acetyl value was determined by the same procedure as employed for heptaacetyl-barbaloin. Required: for 0.4626 Gm. of sample, 41.5 cc. of 0.1 N. NaOH, equivalent to 38.4% acetyl; for 0.5932 Gm., 53.7 cc. of 0.1 N. NaOH, equivalent to 38.8% acetyl; calculated for $C_{20}H_{18}O_9(CH_3CO)_6$: 39.2% acetyl.

Tetrabromo-barbaloin

Test for Carbonyl Group.

Tetrabromo-barbaloin was first prepared by adding a solution of aloin to an excess of bromine water. The derivative precipitated immediately. It was collected, washed, and dried; then crystallized from

diluted alcohol. It gave a melting point of 193°C.

Unlike aloin, Fehling's solution and Tollen's reagent were not reduced. Negative results were obtained with Schiff's test, sodium bisulfite, and phenylhydrazine.

Determination of Arabinose.

Tetrabrome-barbaloin, 2.4 Gm., was refluxed with 250 cc. of 12% hydrochloric acid for 4 hours. The mixture was then distilled and the pentose was determined by the same procedure previously applied to aloin. Yields: I, 0.0082 Gm.; II, 0.0096 Gm.

Stability of Halogen.

A sample of tetrabrome-barbaloin was dissolved in sodium hydroxide solution and heated at 100°C. for 20 minutes. The solution was cooled, acidified with diluted nitric acid, and filtered. The filtrate failed to give a precipitate with silver nitrate solution.

Condensation of Aloin with Aldehydes

Reaction with Formaldehyde.

Four grams of aloin were dissolved in 30 cc. of 20% sulfuric acid, and 10 cc. of 40% formaldehyde was added. The solution was refluxed on a steam bath for about ten minutes, then cooled, and diluted with water. The gummy precipitate was rubbed with a stirring rod until it solidified. The precipitate was removed, washed, and dried. It was dissolved in a diluted solution of sodium hydroxide and reprecipitated by acidifying the

solution with hydrochloric acid. Other attempts to purify the compound through crystallization were unsuccessful.

The condensation product was very insoluble in water, very slightly soluble in ether, slightly soluble in acetone, and soluble in alcohol. It did not appear to give the green fluorescence with sodium borate.

A solution of the product in 12% hydrochloric acid was refluxed for 15 minutes and then distilled. The distillate produced a strong pink color on the addition of aniline acetate.

The formaldehyde condensation product was converted to an acetate in the usual manner with acetic acid anhydride and acetyl chloride. It was dissolved in hot methyl alcohol from which it separated out as a yellow powder, m. p. about 165°C. (not sharp).

The acetyl derivative was soluble in ether, acetone, and benzene. It was brominated by bromine in benzene solution, when the bromine derivative separated within a few minutes. Hydrogen bromide was not evolved.

Reaction with Acetaldehyde.

Two and a half grams of aloin were dissolved in 50 cc. of 20% sulfuric acid and 5 cc. of acetaldehyde was added. The solution was refluxed on a steam bath for 15 minutes, then cooled, and diluted with water. The gummy precipitate which formed was rubbed with a stirring rod until the product solidified. Purification was carried out in the same manner as with the previous compound.

The condensation product was a light brown, amorphous compound, soluble in alcohol, acetone, and ethyl acetate. When distilled with 12% hydrochloric acid, a strong pink color was produced on the addition of aniline acetate to the distillate.

Action of Hydriodic Acid on Aloin

Seven grams of purified aloin were dissolved in 35 Gm. of hydriodic acid, sp. fr. 1.7, rendered colorless by the addition of hypophosphorous acid drop by drop. The solution was allowed to stand at room temperature for 4 days, after which it was carefully neutralized with sodium hydroxide. The solution was kept cold in ice during neutralization; an excess of alkali which would dissolve the precipitate was avoided. The precipitate was rubbed with a stirring rod, removed and washed. It was purified by slow crystallization from a methyl alcohol-water mixture.

The compound was a yellow substance, melting at 118-119°C. It was soluble in alcohol and acetone, and slightly soluble in water and ether. It darkened on exposure to strong light. With sodium borate it failed to produce a fluorescent solution.

Analysis.--Found: I, 46.62% C; 4.43% H; II, 46.56% C; 4.78% H; Calculated for $C_{20}H_{21}O_8I$: 46.52% C; 4.07% H.

Acid Hydrolysis.

A sample of the iodine compound was refluxed for 2 hours with 12%

hydrochloric acid. The pentose was determined as by previous methods.

Found: 0.0060 Gm. pentose from 0.3967 Gm. of sample.

TABLE IV

DERIVATIVES OF ALOIN

Compound	Formula	Per Cent Comp. Observed	Per Cent Comp. Theoretical
heptaacetyl-barbaloin	$C_{20}H_{15}O_9(CH_3CO)_7$	43.1% Ac.	43.0% Ac.
hexaacetyl-barbaloin	$C_{20}H_{15}O_9(CH_3CO)_6$	38.8% Ac.	39.2% Ac.
barbaloin-heptamethylether	$C_{20}H_{15}O_2(OCH_3)_7$	64.8% C 7.1% H 40.7% OMe	64.2% C 7.2% H 43.0% OMe
desoxy-iodo-barbaloin	$C_{20}H_{21}O_8I$	46.8% C 4.8% H	46.8% C 4.1% H

TABLE IV

DERIVATIVES OF ALOIN--continued

Compound	Formula	Per Cent Comp. Observed	Per Cent Comp. Theoretical
*tribromo-barbaloin	$C_{20}H_{19}O_9Br_3$	37.7% C 3.0% H 37.7% Br	37.3% C 3.0% H 37.3% Br
*hexaacetyl-tribromo-barbaloin	$C_{20}H_{13}O_9Br_3(CH_3CO)_6$	43.4% C 3.8% H 26.4% Br	42.9% C 3.8% H 28.8% Br
*methyl-tribromo-barbaloin-pentamethylether	$C_{20}H_{13}O_6Br_3(CH_3O)_5CH_3$	43.0% C 4.0% H 33.3% Br 22.9% OMe	42.9% C 4.3% H 33.0% Br 21.3% OMe
*acetyl-methyl-tribromo-barbaloin-pentamethylether	$C_{20}H_{12}O_6Br_3(CH_3O)_5CH_3(CH_3CO)$	44.1% C 4.1% H 31.4% Br 6.4% Ac.	43.7% C 4.3% H 31.2% Br 5.6% Ac.

*Analyses were taken from the papers of Gibson and Simonsen.

DISCUSSION

Before any rational structure can be advanced for the constitution of aloin, its molecular formula must first be ascertained. Two possibilities were apparent, a C_{20} or a C_{16} formula, respectively based upon a glycosidal or non-glycosidal structure. It was necessary to limit these possibilities to one.

The analyses of tetrabromo-barbaloin ($C_{16}H_{16}O_7Br_3$?), its derivatives, and possibly aloin composed the only support for $C_{16}H_{18}O_7$. Some investigators who regard aloin as a glycoside, fail to accept this halogen compound as a true derivative of aloin, but consider it possibly as a decomposition product. This would indeed be a misconception for the reaction involved in the formation of tetrabromo-barbaloin was found to be instantaneous with the immediate precipitation of the halogen compound. It would appear illogical to assume that 4 carbon atoms could be so readily lost during bromination. Furthermore, the similarity in general structure of aloin and tetrabromo-barbaloin was clearly indicated by at least two reactions. Both substances have been shown to yield methylanthracene(19) on distillation with a zinc dust, and each may yield small amounts of furfural on distillation with hydrochloric acid. These reactions should prove conclusively that the parent structures involved in these compounds are identical.

In spite of a limited number of analyses which may support the C_{16}

formula, the chemical behavior of aloin and tetrabromo-barbaloin did not warrant such a formula for aloin. Leger(25) has shown that the brome compound is oxidized by sodium peroxide to tetrabromo-aloe-emodin. The formation of the latter product from $C_{16}H_{16}O_7Br_2$ would entail mobility of halogen, which admittedly could occur. However, while it may be possible to project this reaction from a C_{16} formula, it would be impossible to derive a logical structure which would explain other reactions as well,--reduction to methylanthracene and hydrolysis or degradation to arabinose.

The C_{16} formula was also disproved by the boron hydrolysis of aloin, in which aloe-emodin anthranol is readily produced. The formation of the latter, $C_{15}H_{12}O_4$, from a C_{16} formula would entail in addition the production of a compound composed of 1 carbon atom: $C_{16} \rightarrow C_{15} + C$? The detection of methyl alcohol was claimed by Simonsen; however, Rosenthaler was unable to verify it. Gardner and Campbell(26) were also unable to demonstrate the presence of methyl alcohol, but reported the presence of a trace of formaldehyde (15 mg. of formaldimethone from 60 Gm. of aloin). The formation of such a small amount of formaldehyde is inconsequential and may have resulted from the decomposition of arabinose by the action of the alkali.

The writer carried out the hydrolysis of aloin with sodium borate and tested for methyl alcohol, formic acid, formaldehyde, carbon dioxide, and carbon monoxide. All results were negative.

The C_{16} formula was further discounted by the writer's monoiodo compound, obtained by the action of hydriodic acid on aloin. Required for $C_{16}H_{19}O_7I$: 42.8% C; 4.2% H; for $C_{16}H_{17}O_6I$: 44.4% C; 3.9% H; Found: 46.6% C; 4.6% H.

The Sinonsen formula has already been considered, however, one more observation should be mentioned. Tetrabromo-barbaloin was found to contain 2 phenolic groups when based on the formula, $C_{20}H_{18}O_9Br_4$. This would correspond to 1.5 phenolic groups on the formula, $C_{16}H_{15}O_7Br_3$, as expressed by the Sinonsen structure. The latter contains only one phenolic group.

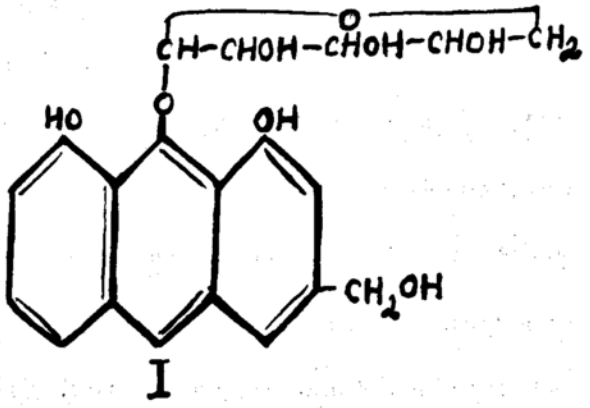
The molecular formula finally decided upon was $C_{20}H_{22}O_9$, which was concluded from the writer's analyses of aloin and the monoiodo compound. This formula was found to be in agreement with tribromo-barbaloin, ($C_{20}H_{19}O_9Br_3$), its derivatives, and barbaloin methylether.

Inasmuch as methylanthracene is produced on reduction, and anthraquinone derivatives on oxidation, aloin must be represented by a methylanthracene structure. In view of such a carbon structure in aloe-emodin anthranol, aloin was at first regarded as a derivative of the anthranol.

It was apparent that one of the hydroxy groups of aloe-emodin anthranol was substituted, as the anthranol was produced by hydrolysis of aloin. In all probability, the hydroxy group in meso position was involved. Thus aloin did not give an immediate fluorescence with sodium borate, but only upon standing. With cupric chloride aloin was not oxidized; whereas aloe-

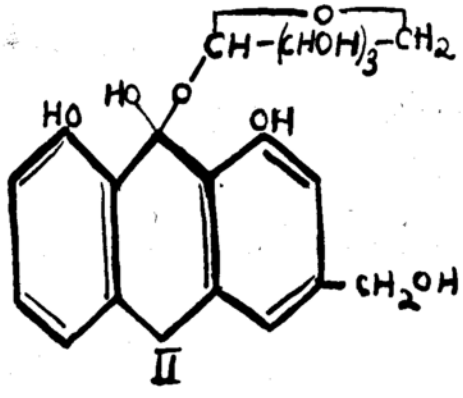
emodin anthranol became readily oxidised. Furthermore, substitution of the meso hydroxyl group would imply two free phenolic groups in aloin. This would be in accord with the finding of two phenolic hydroxyls in tetrabromo-barbaloin.

The pentose derived from aloin was clearly demonstrated by Leger as d-arabinose. As aloin does not contain a carbonyl group, the sugar can only be present in the form of a glycoside, I:

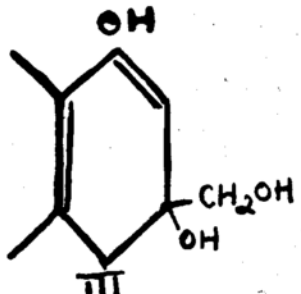


I

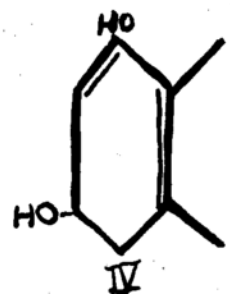
Structure I lacks OH_2 to satisfy the formula $C_{20}H_{22}O_9$ for aloin, therefore, it was obvious that aloin is represented by this structure with H-OH added across one of the double bonds. This led to a number of possibilities, II, III, IV, and V.



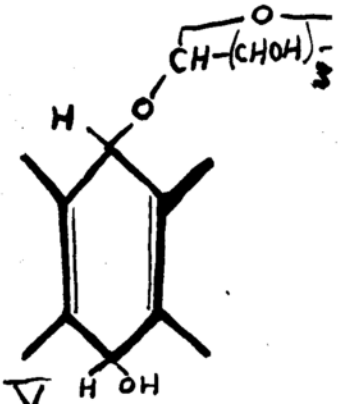
II



III

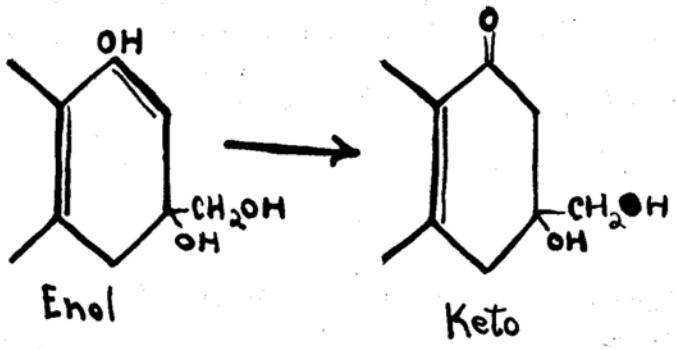


IV



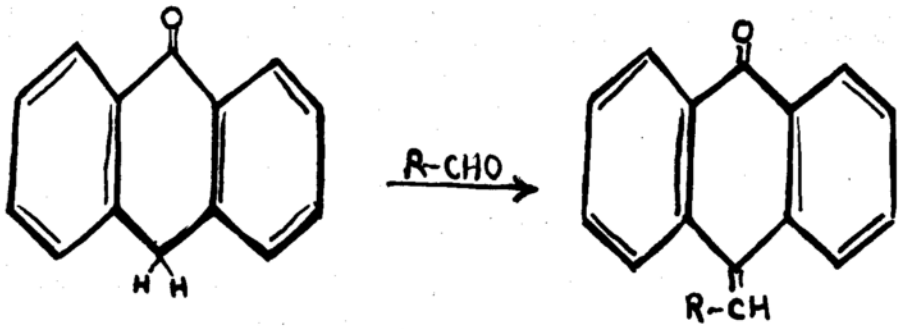
V

Structure III and IV were eliminated on the grounds that these structures represented unstable forms and would be expected to exist as ketonic compounds.



Structure V was also unlikely as it would involve some difficulty in transformation to aloe-emodin anthranol. It would not explain the condensation of aloin with aldehydes.

The most logical choice was structure II which may be considered as an anthrone (anthrone hydrate). As such it would be expected to undergo condensation reactions with aldehydes, a reaction which is not shared by anthranols.

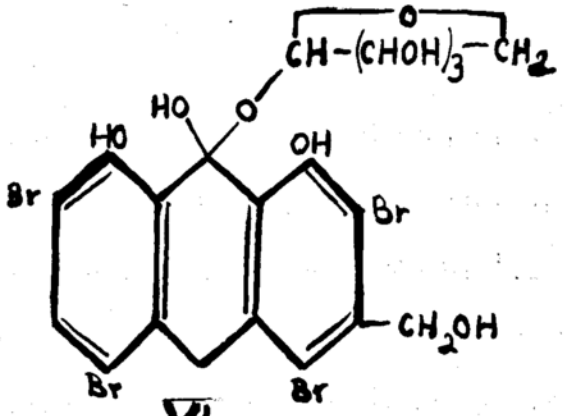


The writer observed that formaldehyde and acetaldehyde condensed readily with aloin, reactions which support the anthrone structure, II, for aloin.

The anthrone formula contains seven hydroxy groups. The methyl ether obtained by Simonsen through repeated methylation with silver oxide and methyl iodide corresponded to heptamethylether-barbaloin. Acetic acid anhydride in the presence of sulfuric acid resulted in the formation of a hexaacetate. The failure of the latter process to produce complete acetylation of aloin may be attributed to the presence of a tertiary alcohol group. However, a heptaacetate apparently was produced by the action of acetyl chloride on aloin.

The anthrone formula was further supported by the halogen compounds, produced by halogenation in aqueous solution. The formation of tetrahalo substitution products would be expected, in contrast to pentahalo derivatives on either Rosenthaler's or Hauser's anthranol formula. Moreover, anthranols are very reactive towards bromine, with either the formation of ms-brom derivatives or oxidation to bi-molecular compounds. Molecular weight determinations, while they cannot be relied upon, did not indicate a dianthryl type of compound for tetrabrom-barbaloin. In addition, the halogen of tetrabrom-barbaloin was found to be very stable towards alkali, in great contrast to the behavior of ms-brom-anthranols which readily lose halogen in the presence of alkali. The following structure may logically be advanced for the representation of tetrabrom-

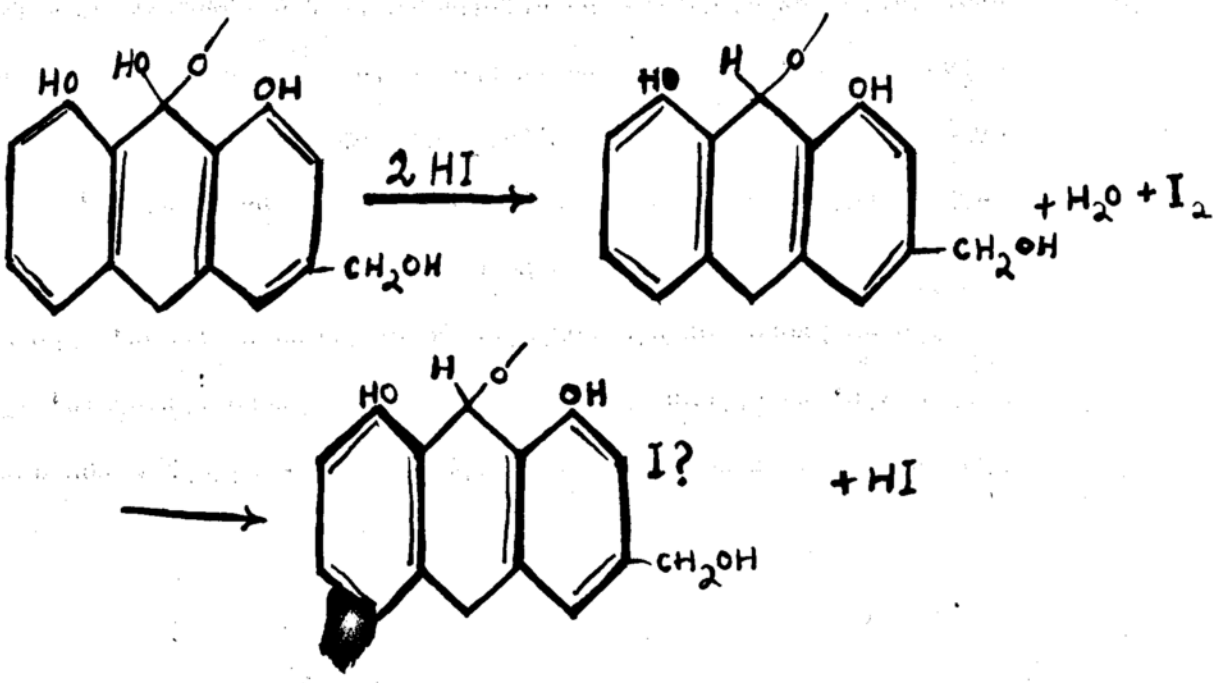
barbaloin:



VI

Structure VI would be in harmony with the finding that diazotised sulfanilic acid coupled with aloin but not with the halogen derivative. The somewhat low values found for halogen on the analyses of tetrabromo-barbaloin may be attributed to the presence of tribromo-barbaloin, $C_{20}H_{19}O_9Br_3$, as an impurity. These aloin derivatives tended to be amorphous and separation through crystallization was not a simple matter.

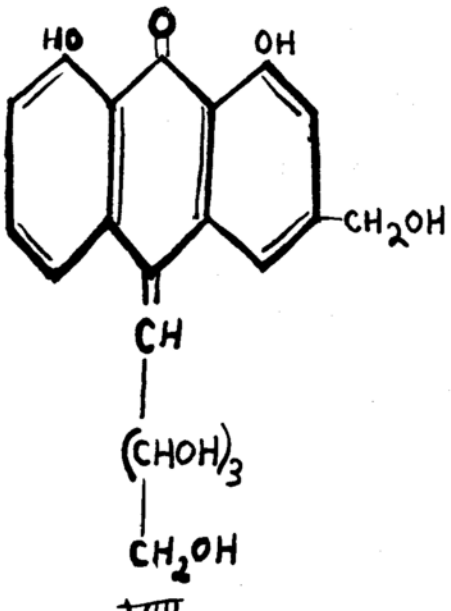
The reduction of aloin by the action of hydriodic acid to form a desoxy-iodo derivative was indicated by the analysis. The formation of this product may be interpreted in the following manner:



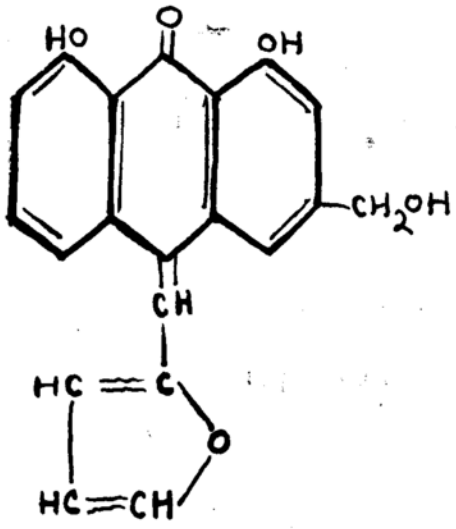
Deoxy-iodo-barbaloin, VII, involves a dihydro-anthranol structure, which would conform with the observation that a fluorescent solution was not produced with sodium borate.

One of the principal objections raised against glycoside formulas advanced for aloin, was the results which followed attempts to estimate ℓ arabinose quantitatively. The determination generally involved hydrolysis and conversion of the pentose to furfural with 12% hydrochloric acid. Furfural was in turn converted to a phloroglucinide which was weighed. The amounts of arabinose accounted for in this manner were exceedingly low, in the order of 2% of the theoretical. These low yields were explainable on the basis of the anthrone structure advanced for aloin.

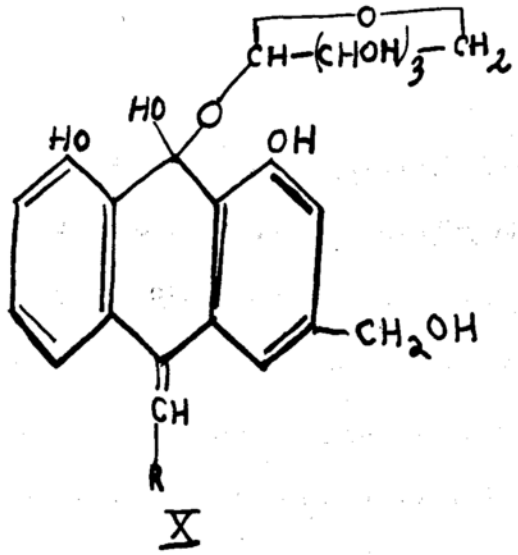
The condensation of aloin with formaldehyde and acetaldehyde as was previously mentioned, apparently constitute a general reaction in which other aldehydes may become involved. Arabinose, an aldopentose, may be anticipated to undergo similar condensation reactions following hydrolysis, with the probable formation of compounds of the following type:



XIII



IX



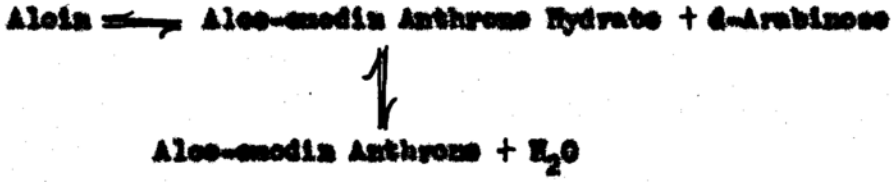
X

When aloin was subjected to preliminary hydrolysis with sodium perborate, and arabinose subsequently determined, greater values were observed(8) for the pentose than were obtained from the action of other non-oxidative hydrolytic agents. In view of what already has been considered, a greater yield of arabinose would even be anticipated from the action of sodium perborate, for as a result of oxidation of the meso carbon atom to produce a quinone, condensation with aldehydes would be prevented. The action of sodium peroxide and other oxidising agents on aloin are known to result in in the formation of aloe-emodin.

In the hydrolysis of aloin with sodium borate, free arabinose was apparently not present in the hydrolysate and the yield of aloe-emodin anthranol was small, not over 10%. Evidently, compounds such as VIII and X were formed during the hydrolysis. The yield of anthranol may be increased, however, by carrying out the hydrolysis in the presence of hydrazine or

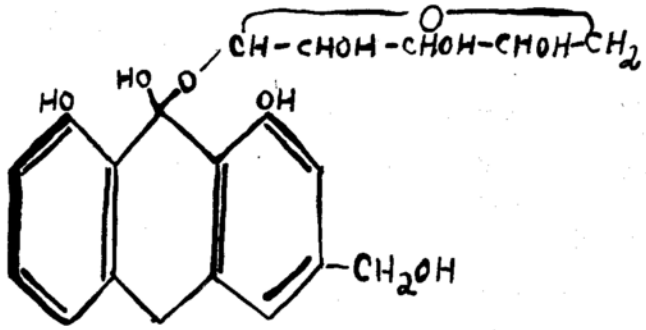
phenylhydrazine. Simonsen(9), who first made this observation, regarded the role of hydrazine as an oxygen acceptor. This conception is unlikely; it is more probable that the amines form addition products with arabinose resulting from hydrolysis, and thereby prevent other condensation reactions.

With a single exception, the chemical properties of aloin were found to be in excellent accord with the proposed formula. Towards acids, aloin behaved in rather an unusual manner for a glycoside compound; it was comparatively stable towards acids, more readily hydrolised by alkali. The instability of aloin in alkaline solutions is not difficult to interpret, as anthrones tend to enolise in the presence of alkali. It is difficult, however to reconcile the fact that a compound with an acetal structure is not readily hydrolised by acids. Nevertheless, an explanation which is partly satisfactory may be advanced on the basis of a reversible reaction during hydrolysis. Accordingly, synthesis would take preference over hydrolysis.



SUMMARY

The molecular formula, $C_{20}H_{22}O_9$, was established for aloin, and the following structural formula was derived:



Accordingly aloin may be considered as an aloe-emodin anthrone hydrate-d-arabinoside.

The chemical behavior of aloin supported the above glycoside structure, viz., formation of d-arabinose and aloe-emodin anthranol on hydrolysis; the presence of seven hydroxy groups, two of which are phenolic; condensation with aldehydes; formation of a tetrabrom substitution product; and absence of a carboxyl group.

The formula was also in agreement with the analyses of a number of derivatives of aloin: heptaacetyl-barbaloin; hexaacetyl-barbaloin; barbaloin-heptamethylether; desoxy-iodo-barbaloin; tribromo-barbaloin; hexaacetyl-tribromo-barbaloin; methyl-tribromo-barbaloin-pentamethylether; acetyl-methyl-tribromo-barbaloin-pentamethylether.

An explanation was advanced for the relative stability of aloin towards acids, on the assumption of a reversible reaction during hydrolysis.

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