A CHEMICAL INVESTIGATION OF *ALOE BARBADENSIS* MILLER*

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Free amino acids, free monosaccharides and total saccharides released upon hydrolysis, sterols, and triterpenoids of the leaves of *Aloe barbadensis* Miller leaves were determined. Some seventeen amino acids, D-glucose, and D-mannose were present in the water-soluble fraction. Cholesterol, campesterol, β-sitosterol, and lupeol were found in substantial amounts in the lipid fraction. An unknown(s) alkaloid was detected using Dragendorff's reagent.

INTRODUCTION

Since the earliest days of recorded history, man has made use of *Aloe* plants (1, 2, 3). There are several references to aloes in the Bible (4) but since it was then used as a perfume or incense, identification with the modern species of *Aloe* (family *Liliaceae*), which are not known for their aromatic properties, is doubtful (5). The one used in this study is *Aloe barbadensis* Miller (Figure 1), commonly called *Aloe vera;* the juice of this plant has an unpleasant odor and taste. This species is native to the Mediterranean region (or var. *chinensis* to India), but is now widely distributed in southern parts of North America, Europe, and Asia.

The Chinese were among the earliest people who used the plant for its medicinal qualities (6), but it was considered valuable throughout the Middle Ages for a variety of ills. Even today there is considerable use of *A. barbadensis* in folk medicine in the southernmost United States, and some cosmetics and patent medicines generally found on the market are prepared from the gel in the leaves (Figure 2) and from the juice. Over a hundred thousand plants have been grown at one time in Florida, Texas, and Mexico for market, and it has been estimated that annual sales in this country amount to millions of dollars (7).

A number of paramedical publications extol its ability to promote the healing of burns and other cutaneous injuries and of ulcers of mucous membranes; this literature has been reviewed by Gjerstad and Riner (7). Many housewives are reported to grow *A. barbadensis* as a kitchen plant just to provide a home treatment for minor skin injuries. A preparation made from it has been patented as a topical medication for burns (8).

There are contradictory claims on the bacteriostatic activity of *Aloe* leaves and extracts (7, 9), but some five scientific reports attribute improved healing of burns to their treatment with *A. barbadensis* products (7). These have also been found effective against peptic ulcers (10), skin disorders (11, 12), and infections treated in veterinary medicine (13).

Aloes have long been recognized by pharmacopoeias over the world (e.g., 14, 15) as a purgative drug; one variety, called Curacao aloes, is the dried juice of *A. barbadensis.* This use of aloes led to studies of their composition. In 1956 paper chromatography showed the presence of anthranol (I), aloe-emodin (II), and chrysophanic acid (chrysophanol) (III) (16) (Figure 3). However, when the juice of fresh *Aloe* leaves was studied in a nitrogen atmosphere, aloin (barbaloin) (IV) and *p*-coumaric acid (V) but no aloe-emodin was found (17); presumably the latter was an artifact produced by air oxidation. A commercial sample of aloes (*A. barbadensis*) contained aloesin (VI) (18); in another one the principal constituent was barbaloin, but some free aloe-emodin and isobarbaloin were present (15). A thin-layer chromatographic study of 22 species of *Aloes* (19) showed that 12 species contain flavanoids, hydroxyanthraquinones, and coumarin.

*Journal article 3083, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.
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The carbohydrates of *Aloe* juice have been reported to be glucose (20) and a polyuronide composed of (a) a polyose (molecular weight up to about 2.75×10^5) containing glucose and mannose and (b) hexuronic acids such as glucuronic, mannuronic, and galacturonic acids (8). Later work confirmed that hydrolysis gives glu-

FIGURE 1. Middle: Mature *Aloe barbadensis* plants in flower. Top Right: Close-up photograph of the *Aloe barbadensis* flowers (greenish yellow in color). Top Left: Detailed view of the upper part of the flowering stalk.

cose and mannose, as well as traces of galactose, arabinose, and xylose, but found no uronic acids (21).

The juice is of course mostly water (99.52%); the lyophilized and dried product contained a variety of functional groups as shown by infrared spectroscopy (IR), 2.5% protein by Kjeldahl analysis, free hydroxyproline, histidine, and cystine by thin-layer chromatography (TLC), and after hydrolysis the amino acids shown in Table 1 (20). Bradykininase activity is present in *Aloe arborescens* Miller (22), but the existence of any anti-inflammatory action *in vivo* remains to be shown. The effects of prednisolone, indomethacin and *Aloe* gel on tissue culture cells showed that *Aloe* gel was less toxic to HeLa cells and rabbit kidney cells than prednisolone or indomethacin; however, at a serial dilution of 5×10^{-1}, the *Aloe* gel solution was completely toxic to both cell lines (23).

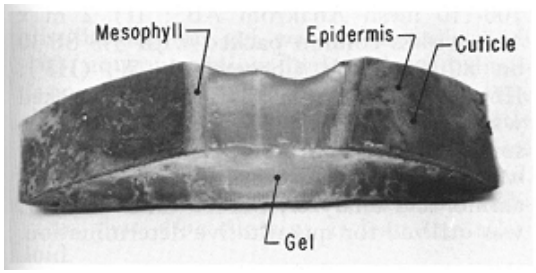


FIGURE 2. Photograph of a 1-inch section of an *Aloe* leaf which has been partly cut to show the various layers. The leaf was originally 24 inches long and approximately 4 inches wide at the base.

Thus, as in the case of many other folk medicines, the efficacy of *A. barbadensis* remains uncertain, and some potentially physiologically active constituents are still unidentified. The present paper reports the occurrence of free amino acids, free monosaccharides and those produced by hydrolysis, sterols, and triterpenoids in *Aloe barbadensis* leaves (24).

MATERIALS AND METHODS

Plant material

Aloe barbadensis Miller plants were grown from young plants (obtained from Hilltop Gardens, Lyford, Texas) to maturity in the Oklahoma State University horticultural greenhouse. For positive identification of this species of *Aloe,* a specimen in bloom was sent to the Plant Science Research Division of the United States Department of Agriculture, Beltsville, Maryland, where it was identified as *Aloe barbadensis.*

Extraction and fractionation

Since there were reports that severed *Aloe* leaves rapidly lose their medicinal properties (6, 9) the material used was either fresh or lyophilized and stored at –15 C. *A. barbadensis* leaves (35 g equivalent of dry material) were macerated and extracted with water-acetone (1:1) and then with acetone at room temperature. The combined extracts (2:1) were concentrated in a rotary evaporator (35 C) and the acetone-free residue was extracted three times with diethyl ether (250 m*l* each time). The lipid phase was taken to dryness and the residue refluxed with 10% potassium hydroxide in 50% ethanol (50 m*l*) for 3 hr. This hydrolysate was extracted with diethyl ether and the concentrated organic phase (0.25 g) was chromatographed on a silica gel column (1.5 × 35 cm), eluted with benzene: diethyl ether (8:2), and collected in 10-m*l* fractions. The resulting 20 fractions were assayed by thin-layer chromatography (TLC) (see chromatography sec-

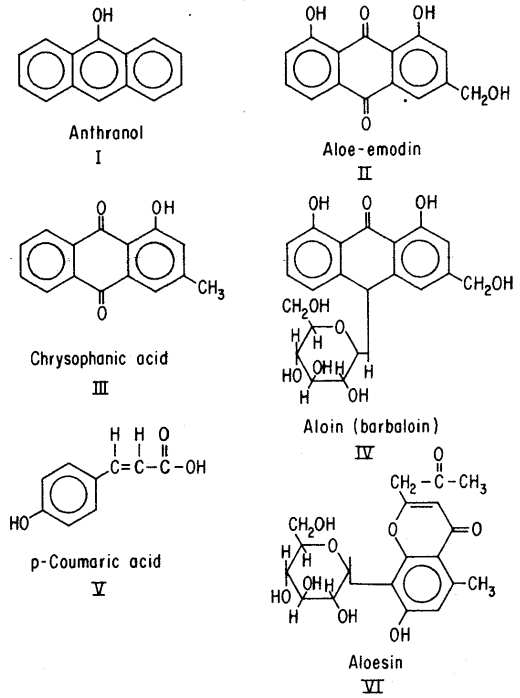


FIGURE 3. Structural formulas of compounds isolated from *Aloe barbadensis.*

tion: silica gel, solvent 4, chromogenic reagent b) and recombined to give triterpenoid and sterol fractions.

The aqueous phase (250 m*l*) was concentrated under reduced pressure, clarified by centrifugation, and passed successively through Dowex-50 W (50-100 mesh, × 8, H^+) (3.5 × 35 cm) and Amberlite IRA-400 (50-100 mesh, × 8, OH^-) (4 × 40 cm) columns. The aqueous eluate was taken to dryness and constituted the "neutral fraction." The "cationic fraction" was obtained by treatment of the cation exchange resin with 2*N* NH_4OH (2 *l*).

Steam distillation

The tough outer portion consisting of the cuticle, epidermis, and mesophyll (see Figure 2) was removed from the leaves of mature plants brought from Texas. The green outer portion and the colorless inner part as well as the "stalk" (that portion of the plant above the ground remaining after the leaves had been removed) and the roots were steam distilled, each separately. The distillation was allowed to proceed as long as the condensate had a definite odor; this was approximately 5 hr.

The condensate from the steam distillation was saturated with sodium chloride, extracted with diethyl ether, dried with anhydrous sodium sulfate and the ether was reduced in volume using a stream of nitrogen.

Chromatography and mass spectrometry

Precoated silica gel plates were used for TLC. The solvent systems were: 1) 1-butanol:acetic acid:water (12:3:5); 2) phenol:water (3:1); 3) 1-butanol:acetone:water (7:2:1); 4) benzene:diethyl ether (8:2); 5) chloroform:methanol (5:1); 6) hexane:acetone:ethanol (40:10:4). Solvents 1 and 2 were used in that order to develop two-dimensional thin-layer chromatography (2-D TLC). Chromogenic reagents were: 0.1% ninhydrin in acetone; b) 1% ceric sulfate $Ce(SO_4)_2$ in 2*N* H_2SO_4; c) 0.05% Rhodamine 6G in acetone; d) Dragendorff's reagent (25). GLC was performed using a modified Barber Colman Model 5000 gas chromatograph with flame ionization detector (30) and He as carrier gas. The following columns were used: I) 3 m × 4 mm glass column packed with 4% OV-101 on 100-110 mesh Anakrom ABS; II) 2 m × 4 mm glass column packed with 3% SE-30 on 80-100 mesh Chromosorb W (HP); III) 1.5 m × 4 mm glass column packed with 3% OV-25 on 80-100 mesh Chromosorb W (HP). A prototype of the LKB-9000 was used for GLC-MS (26). A Beckman amino acid analyzer, Model 120C (AAA), was utilized for quantitative determination.

Examination of fractions

Cationic fraction. Amino acids present in this fraction were identified and quantitated by 2-dimensional TLC and by AAA, conjunctively. Glutamine and asparagine were determined by measuring the increase of parent acids following hydrolysis (1*N* HCl /3 hr in N_2) (27).

Neutral fraction. A dry aliquot (10^{-2}) of this fraction was trimethylsilylated (TMS) according to Sweeley *et al.* (28). In order to avoid tailing, the reagents were removed before GLC by extraction with chloroform. Column I was used isothermally at 190 C, flow rate 50 m*l*/min. Peaks were identified by comparison with standards as D-mannose (one peak) and D-glucose (two peaks). Results were confirmed by TLC of the free sugars (silica gel, solvent 3, chromogenic reagent b). Determination of both TMS-sugars (whose molar detector constants were experimentally found to be 1.0) was achieved using *α*-D-xylose as an internal standard. The peak area was calculated triangulation (retention time relative to that of TMS-*α*-D-xylose : TMS-*α*-D-mannose 1.38; TMS-*α*-D-glucose 1.78; TMS-*β*-D-glucose 2.35).

A sample of the transparent gel-like portion of the *Aloe* leaf (Figure 2) which had been freed of all the green material was lyophilized and a sugar analysis was performed. The lyophilized sample was hydrolyzed in 2*N* H_2SO_4 for four hr at 100 C. The hexosamines were separated from the neutral sugars on a Dowex H^+ column and the neutral sugars were determined by the method of Lee, McKelvy, and Lang (29).

Steam distillation. The blended gel changed from nearly colorless to a very deep pink during distillation. The color change was not due to a change in pH, for the pH remained at 5.0 during the distillation. Then the distillation mixture was made acidic to pH 1.0. Following the acid

distillation, the gel was made basic with 10% aqueous sodium hydroxide and distillation was continued. The gel became very dark in color and appeared to be partly digested by the base, while the distillate had an odor similar to that of burnt wood. The Dragendorff's positive reaction indicated that *Aloe* contains at least one alkaloid.

Repeated attempts to extract material that gave a Dragendorff's positive reaction were made but no positive results were obtained. After spraying with Dragendorff's reagent, some compounds present in a methanol extract or a dichloromethane extract at the origin of the TLC plate (solvents 5 and 6) showed a slowly developing blue coloration. This may mean that the Aloe develops a light-sensitive compound when sprayed with Dragendorff's reagent or it may contain a complex alkaloid that is set free upon distillation.

Triterpenoid fraction. Fraction 3 to 5 were chromatographed by preparative TLC (silica gel, solvent 4, chromogenic reagent b). The band with R_f 0.53 was eluted with diethyl ether; this gave 12 mg of a mixture, the major component (96%) of which was identified as lupeol by NMR and GLC-MS (Column III, operating isothermally at 220° C, flow rate 30 m*l*/min). The major mass spectral fragments agree with the standard values observed and reported (30, 31). The NMR spectrum proved particularly informative with respect to the isopropenyl side chain in ring E. The methyl group at C-29 appeared as a singlet at δ 1.66 (32). The two olefinic protons resonated at 4.56 and 4.68 δ (J_{gem} 1.8 Hz).

Sterol fraction. The sterols were in fractions 7 to 15. Crystallization from methanol gave 30 mg of a mixture with m.p. 132-134 C. The IR spectrum (KBr) gave strong absorption bands at γ max. 3400, 2930, 1460, 1375, 1060, 1025 cm^{-1}, GLC-MS (column II isothermally at 245 C, flow rate 30 m*l*/min) showed the mixture to be composed of cholesterol (M^+ 386) (7%), campesterol (M^+ 400) (6%) and β-sitosterol (M^+ 414) (87%). The spectra were consistent with those of standard samples and with the published fragmentation patterns of sterols (33).

RESULTS AND DISCUSSION

The total water-acetone extracts were separated into lipids and water-soluble fractions. Water-soluble compounds were divided into a "cationic fraction" and a "neutral fraction" by using ion-exchange resins. Lipids were hydrolyzed and the nonsaponifiable fraction was separated by column chromatography into triterpenoid and sterol fractions.

All fractions were examined by appropriate methods, and individual constituents were identified.

Cationic fraction

Amino acids were identified and determined by 2-D TLC and AAA. No unusual amino acids were found. Table 1 shows that arginine is relatively abundant (approx. 20% of total amino acids).

Gjerstad (20) reported that expressed juice from *Aloe barbadensis* contained glutamic acid and aspartic acid as the major amino acids. Our results for aspartic and glutamic acids plus their corresponding amides yielded (combined: Asp 237 + Asn 343 = 580 μmol/100 g dry leaf and Glu 294 + Gln 141 = 435 μmol), which confirms and extends Gjerstad's results. Thus our analysis shows that the amino acid in highest concentration is arginine, followed by asparagine, glutamic acid, aspartic acid, and serine.

TABLE 1. *Free amino acids and monosaccharides in* A. barbadensis *leaves.*

Amino Acid[a] or Sugar	Level in dry leaves, (μ mole/100 g)
Aspartic acid	237
Glutamic acid	294
Serine	224
Threonine	123
Asparagine	344
Glutamine	141
Proline	29
Glycine	67
Alanine	177
Valine	109
Isoleucine	65
Leucine	53
Tyrosine	28
Phenylalanine	43
Lysine	53
Histidine	15
Arginine	449
d-Glucose	$21.2 \cdot 10^3$
d-Mannose	$8.3 \cdot 10^3$

[a]All amino acids listed were also reported by Gjerstad (20) except asparagine and glutamine, but only after hydrolysis; he also found hydroxyproline, cysteine, and methionine.

Neutral fraction

Following analysis by TLC and GLC two monosaccharides were identified, D-glucose and D-mannose. The former is the most common sugar, the most abundant of all organic compounds, and is almost universally found free. Free D-mannose, conversely, previously has been reported only in the peel of oranges (*Citrus bigaradia*) and in the plant *Cornynocarpus laevigata* (34).

Since differences in the composition of a polysaccharide isolated from *Aloe barbadensis* have been reported by different investigators (8, 21), a sugar determination was performed on the hydrolyzed lyophilized gel. As shown in Table 2, Farkas found mannose and glucose only (the molar ratio was not stated) while Segal, Taylor, and Eoff (21) found mannose and glucose in a ratio of 9-10:1, and traces of arabinose, galactose, and xylose. On the other hand, Farkas reported hexuronic acids while Segal, Taylor, and Eoff could find none. In the present investigation, no attempt was made to isolate either a polyuronide or a particular polysaccharide before the analysis, but rather a lyophilized sample of the whole gel was subjected to analysis. (It was found that 88% of the weight of the gel was lost during lyophilization.) If several different polysaccharides had been present, these also would have been measured by this technique. The results obtained are shown in Table 3; the hexuronic acid content of the gel was not measured. Mannose and glucose were found in a molar ratio of 5:4, and trace amounts of xylose, rhamnose, galactose, and either arabinose or fucose were also found.

TABLE 2. *Sugars of* Aloe barbadensis.[a]

Compound	Found by Farkas (8)	Found by Segal, Taylor and Eoff (21)	Found in this laboratory
Arabinose	—	+	+[b]
Galactose	—	+	+
Glucose	+	+	+
Mannose	+	+	+
Rhamnose	—	—	+
Xylose	—	+	+
Hexuronic Acids	+	—	[c]

[a]The symbol + indicates that the compound was found in the *Aloe* leaves and the symbol — indicates that it was not found.
[b]Arabinose could not be distinguished from fucose. It may be noted that Gjerstad (20) also reported an aldopentose, R_f 0.18.
[c]Hexuronic acids were not sought.

TABLE 3. *Sugar content of* Aloe *gel as determined in this laboratory.*

Sugar	Sugar in whole gel (μmole/g)	Total Sugar in lyophilized residue (%)
Arabinose	4.23[a]	4.7[a]
Galactose	3.60	4.3
Glucose	31.3	37.7
Mannose	39.4	47.5
Rhamnose	1.27	1.5
Xylose	4.44	4.4

[a]Arabinose could not be distinguished from fucose.

Triterpenoid fraction

This fraction, purified by preparative TLC, was analyzed by NMR and GLC-MS. The only triterpenoid present in substantial quantity, as shown in Table 4, was identified as lupeol [1]. This report confirms that lupane is one of the basic carbon skeletons found in *Monocotyledoneae* (35).

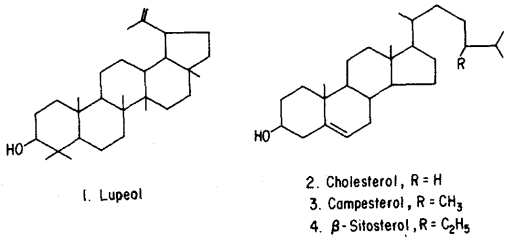
Sterol fraction

The sterols were examined by means of melting point, IR, and GLC-MS. Cholesterol [2], campesterol [3], and β-sitosterol [4] were present.

Campesterol and β-sitosterol are typical plant sterols. Cholesterol has, for many years, been considered a typical animal sterol. In 1959, however, it was discovered

TABLE 4. *Sterols and triterpenoids in* A. barbadensis *leaves.*

Sterol or Triterpenoid	Level in dry leaves (μmole/100 g)
Cholesterol	10.8
Campesterol	12.4
β-Sitosterol	148.0
Lupeol	66.1



1. Lupeol
2. Cholesterol, R = H
3. Campesterol, R = CH_3
4. β-Sitosterol, R = C_2H_5

FIGURE 4. Structures of triterpenoids and sterols from *Aloe barbadensis* Miller.

in red algae (36) and subsequently its presence was shown in microorganisms and higher plants (37).

Steam distillation

A comparison of the spots resulting from thin-layer chromatography of the volatiles from the gel of the inner part of the *Aloe* leaves, the outer layers of the leaves, the stalk, and the roots as seen under visible light and 366 nm light and after spraying with Dragendorff's reagent is seen in Figure 5.

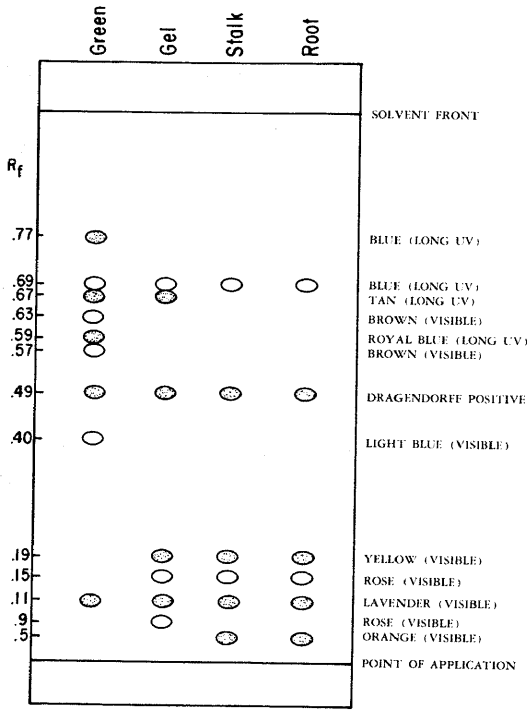


FIGURE 5. A comparison of the steam volatiles from the inner leaf gel, the outer leaf layers ("green" above), the stalk, and the root of *Aloe barbadensis* as seen on a thin-layer chromatogram developed in hexane:acetone:ethanol (40:10:4).

ACKNOWLEDGMENT

This investigation was supported in part by Merrick Foundation and by National Science Foundation Grant BMS 70-00583-A02. The authors wish to thank Dr. O. C. Dermer for helpful suggestions, Mrs. Carol Ahmed, who assisted with the preparation and presentation of this paper, Dr. Ta-Hsiu Liao and Mrs. Linda McKenzie for use of the amino acid analyzer, and Cathy Waller and Steve Young for assistance in obtaining the mass spectra. Acknowledgment also is made of the help given by Dr. Frederick G. Meyer and Mr. Harold F. Winters in the identification of these plants.

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