

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), aloë-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 aloë-emodin was found (17); presumably the latter was an artifact produced by air oxidation. A commercial sample of aloes (*A. barbadensis*) contained aloësin (VI) (18); in another one the principal constituent was barbaloin, but some free aloë-emodin and isobarbaloin were present (15). A thin-layer chromatographic study of 22 species of *Aloes* (19) showed that 12 species contain flavonoids, hydroxyanthraquinones, and coumarin.

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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 ml/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 ml/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 non-saponifiable 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 μ mol/100 g dry leaf and Glu 294 + Gln 141 = 435 μ mol), 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	419
D-Glucose	21.2 \times 10 ³
D-Mannose	8.3 \times 10 ³

^aAll amino acids listed were also reported by Gjerstad (20) except asparagine and glutamine, but only after hydrolysis, he also found hydroxyproline, cysteine, and methionine.

	Moist. %	Ash %	Total Carb. %	En. Cal Val. C/100g	Beta Car. 100/100g	Vit. C %	Chol. %	Calc %	Iron %	Magn %	Potass. %	Sodl %	Total Diet. Fib. %	Pro- tein %	Calori From Fat %	Sat. Fatty Acids %	Fat %	Total Sugar %	Fruct %	Dextr %	Sucro %	Malto %	Lactis %
Decl. 1:1	99.1	0.16	0.5	3	<20.	<1.	<2.	52.3	<0.2	3.5	8.6	33.2	0.2	0.2	<1.	<0.1	<0.1	<1.	<0.1	<0.1	<0.2	<0.3	<0.5
Not Decl. 1:1	99.1	0.21	0.4	3	<20.	<1.	<2.	48.5	<0.2	3.7	4.7	37.1	<0.1	0.2	<1.	<0.1	<0.1	<1.	<0.1	0.2	<0.2	<0.3	<0.5
Decl. WL 1:1	97.2	0.43	2.2	10	<20.	<1.	<2.	71.4	<0.2	22.6	115	28.8	<0.1	0.2	<1.	<0.1	<0.1	<1.	0.1	0.6	<0.2	<0.3	<0.5
10:1	94.5	1.79	3.5	15	<20.	<1.	<2.	412	<0.2	25.9	30.6	280	<0.1	0.2	<1.	<0.1	<0.1	1	0.2	1.1	<0.2	<0.3	<0.5
40:1	80.9	5.5	13	55	<20.	<1.	<2.	1,230	0.5	92.1	106	960	0.5	0.4	2	<0.1	0.2	4	0.7	3.6	<0.2	<0.3	<0.5
40:1 WL	78.8	3.62	17	71	<20.	<1.	<2.	570	0.8	146	1,010	107	0.4	0.4	2	<0.1	0.2	6	1.4	4.1	0.5	<0.3	<0.5
100:1	3.62	14.9	80.4	328	<20.	<1.	<2.	2,810	1.9	300	529	2,360	0.6	0.7	3	<0.1	0.3	20	2.2	16.2	<0.2	1.4	<0.5
100:1 WL	2.09	10.7	86	351	<20.	<1.	<2.	1,420	1.7	443	2,960	272	2.5	0.9	3	<0.1	0.3	16	3.9	9.4	1.1	1.3	<0.5
200:1	7.43	32.6	58.4	243	<20.	<1.	<2.	4,140	2.8	628	1,320	4,930	3.5	0.9	6	<0.1	0.6	40	13.1	26.5	<0.2	<0.3	<0.5
200:1 WL	4.84	22.5	68.7	305	<20.	3	<2.	3,290	5.3	863	5,980	588	8.1	1.1	1.1	26	<0.1	2.9	28	5.3	21	1.6	<0.5
200:1 ARF Std.	6.61	43	44.5	213	<20.	440	<2.	7,160	6	481	806	4,680	15.3	3.6	21	<0.1	2.3	12	2.5	9.3	<0.2	<0.3	<0.5

* Calories/100g

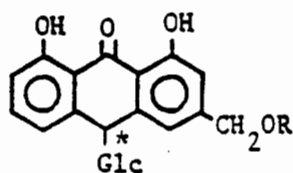
** mg/100g

***gs/100g

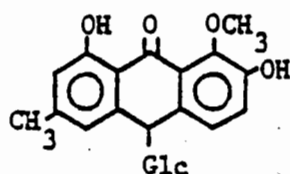
Fig. 1 アロエ及び Aloe 属植物より単離されている phenol 類

I. anthracene 誘導体

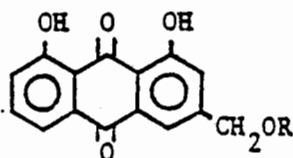
- | | R | [基源] |
|------------------|----------------|-----------------|
| (1) barbaloin | : H | G ⁴⁾ |
| (2) isobarbaloin | : H | G ⁴⁾ |
| (3) aloinoside A | : α-L-rhamnose | I ⁵⁾ |
| (4) aloinoside B | : α-L-rhamnose | I ⁵⁾ |



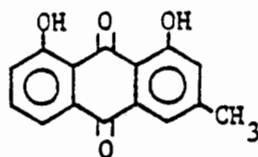
- | | | |
|------------------|-------|-----------------|
| (5) homonataloin | | H ⁶⁾ |
|------------------|-------|-----------------|



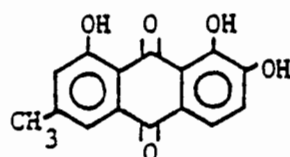
- | | R | |
|---------------------------------|----------------|-----------------------|
| (6) aloe-emodin | : H | E, F, G ⁷⁾ |
| (7) aloe-emodin
L-rhamnoside | : α-L-rhamnose | E, F, G ⁷⁾ |



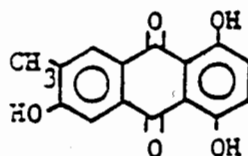
- | | | |
|------------------|-------|-----------------|
| (8) chrysophanol | | D ⁷⁾ |
|------------------|-------|-----------------|



- | | | |
|--------------------|-------|-----------------|
| (9) nataloe-emodin | | C ⁷⁾ |
|--------------------|-------|-----------------|



- | | | |
|-----------------|-------|-----------------|
| (10) rhababeron | | G ⁷⁾ |
|-----------------|-------|-----------------|



* (1)と(2)及び(3)と(4)はそれぞれC₁₀の立体異性体である。

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