

A SAPONIN FROM AGAVE LECHUGUILLA TORREY.

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Agave lechuguilla Torrey, known among the Mexicans as "lechuguilla" or "amole de lechuguilla," grows abundantly on the limestone highlands of western Texas and along the Rio Grande as far east as Presidio, extending into New Mexico and Mexico, where, according to Michotte (1), it grows wild in abundance in the states of Tamaulipas, Chihuahua, Cahahulla, Hidalgo, and Nuevo Leon. It is of interest to note that different forms of agave have been called "*lechuguilla*." Watson (2) refers to "*lechiguilla*" or "*lechuguilla*" as the native name of *Agave guttata* and *Agave variegata*. According to Kew Bulletin (3), these species belong to quite another group, different from *Agave lechuguilla*. The statement is made that

"We may look upon *Agave lechuguilla* Torrey, *Agave poselgerii*, Salm-dyck, and *Agave heteracantha*, Zucc., as synonymous names representing one and the same plant."

This conclusion is evidently based on Engelmann (4) and Baker's (5) classification. In discussing *Agave lechuguilla* Torrey and its relationship, Mulford (6), states in conclusion:

"Though this plant certainly shows affinities with *Agave heteracantha* Zucc. and *Agave poselgerii* Salm-dyck, it differs from them in having a more stiffly suberect and one-sided habit and in never developing a broad pale band down the face of the leaf. The group to which all these and a number of related forms belong should receive careful study and comparison. Our plant may prove to be a variety."

She mentions also that the specific name was originally printed "*lecheguilla*" through an error and should be "*lechuguilla*." Michotte (1) refers to "*lechuguilla*" as "*Agave (littaea) multilineata*

Baker, *Agave heteracantha* Zuccar, c'est lechuguilla du Mexique," which classification is obviously erroneous, as Baker (5) specifically stated, "*Agave (littaea) multilineata* Baker is the same as *Agave heteracantha*, Hort. Angl. not Zucc." Rose (7) states that *Agave lechuguilla*

"has been confused with *A. heteracantha*, from which, although the two are closely related, it appears to be distinct. . . . Our herbarium seems to show at least four good species of the heteracantha group. . . . I should not hesitate to describe some of them as new if I understood what is really the type of *A. heteracantha* Zucc. and *A. poselgerii* Salm. I have the type of *A. lechuguilla* and have seen the description of *A. heteracantha*, but the latter answers to no specimens we have."

The material used in this investigation was obtained from Uvalde, Texas, and was collected in April, 1916. It consisted of the rootstocks and short stems, and in most cases the leaves were attached. In identifying this material as *Agave lechuguilla* Torrey, we have received the assistance of Prof. William Trelease, of the University of Illinois, and of Mr. L. H. Dewey and Mr. E. O. Wooton, of the United States Department of Agriculture, who have furnished a number of photographs, some of which show the general growth of the plant and the characteristic morphological structures (Fig. 1).

Agave lechuguilla is known to contain a substance which foams freely with water (8); in fact, the name "amole de lechuguilla" refers to this soapy character. The plant is valued, however, chiefly on account of the fibers which are abundant in the leaves and possess great strength and durability. The fiber is called Tampico ixtle and istle, or, according to more recent information, Tula ixtle (9). A saponin is found throughout the plant, in the rootstocks as well as in the leaves, which, after the fibers have been removed, are used locally as soap substitutes. It is said that shampoo mixtures are also prepared from the rootstocks of the agave. As far as we have been able to learn the saponin has not been isolated hitherto.

An almost colorless and practically ash-free saponin, which, however, could not be crystallized, has now been isolated from the alcoholic extract of the rootstock.

The hydrolysis of this saponin yielded a pro-sapogenin, a well crystallized end-sapogenin melting at 183.5°C., and two sugars. The end-sapogenin proved to be identical with a sapogenin previously obtained from a saponin from *Yucca filamentosa*. One of the sugars, which was isolated in crystalline form, was shown to be galactose; the other was identified as glucose by means of its phenyl- and para-bromophenylosazones and by its specific rotation.



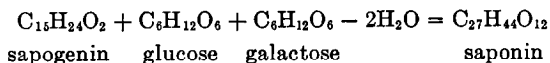
FIG. 1. Leaves of *Agave lechuguilla* Torrey showing the characteristic margin.

Although the saponin gave positive pentose tests with Bial's reagent and yielded furfural on distillation with concentrated hydrochloric acid, the authors could get no further evidence of the presence of a pentose. It is probable that the results of these tests were due to the galactose which is known to give such pentose reactions.

Numerous ultimate analyses were made of the saponin and its sapogenin and the molecular weight of these compounds was determined. Accurate analyses of the saponin were difficult to

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obtain because of its hygroscopic character. The presence of a small quantity of moisture which could not be removed at 110°C. would, of course, tend to lower the carbon content. Heating to a higher temperature was not safe as it caused decomposition which was indicated by a darkening of the compound. The molecular weight determinations show that the composition of the saponin does not agree with Kobert's general formula (10) for saponins, $C_nH_{2n-16}O_{28}$. Even if Kobert's formula were altered to $C_nH_{2n-8}O_{14}$, the results obtained on the hydrolysis of the saponin cannot be made to agree with it. The sapogenin is a well defined crystalline compound with a sharp melting point and can readily be purified from the crude sapogenin by crystallization from alcohol. The results of the ultimate analysis and the molecular weight determination of this compound agree well with the formula $C_{15}H_{24}O_2$. It seems best, therefore, to deduce the formula of the saponin from its products of hydrolysis in the following manner:



EXPERIMENTAL.

Histochemical Experiments.

The saponin was found throughout the plant, in the roots, rootstocks, short axis, and leaves. It was most abundant in the basal parts of the plants, where the fibrovascular bundles were numerous and not especially fibrous and the tracheæ or tracheids formed an essential part of the bundles. A section through the rootstock or axis or even through the bases of the leaves, particularly in the fresh state, when moistened with concentrated sulfuric acid, revealed to the unaided eye the color changes which are characteristic of saponins. This reaction was especially distinct within the bundles, which at first became yellow and then slowly turned pink and reddish white.

Under the microscope the saponin masses were found most easily in the air-dried material. Here they appeared as amorphous, usually transparent, yellowish to yellow-brown films covering the walls or filling the cells of tracheæ and tracheids. They could also be found in the parenchymatous and sclerenchymatous

tissue surrounding these elements. In the fibers of the leaves the tracheæ form only a small part and are frequently scarcely visible without staining. Saponin may, however, be found in the tracheal veins, distributed rather abundantly in the parenchymatous tissue surrounding the fibers. From these observations it is believed that the saponin occurs in the sap of the cells. Since the saponin contains 2 molecules of sugar, it may be used as food material in the metabolism of the plant. The fact that the saponin is strongly hygroscopic suggests that it may also serve to retain moisture in the plant tissue during the long periods of draught prevailing in desert regions.

The histochemical studies were somewhat complicated by the fibrous character of the leaves and the serious decay of the rootstocks which usually occurs in grown agave plants. Since the saponin was easily soluble in water and in 95 per cent or more dilute alcohol, the plant material to be tested could not be immersed in these liquids. Unfortunately, lead acetate (neutral and basic), barium hydroxide, and cholesterol, which usually precipitate saponins, could not be used, as they caused no precipitation of saponin in this case. Ether, absolute alcohol, and olive oil were used for immersing the sections prepared in the experiments to locate the saponin. None of these liquids, however, were altogether satisfactory. Ether evaporates rapidly, absolute alcohol dissolves the saponin slowly, and olive oil is not readily removed from the tissue, making it difficult to complete the test.

The use of a suspension of blood corpuscles, recommended in a previous paper (11), was again tried, but gave satisfactory results only in the roots. Here calcium oxalate, although present in other parts of the plant, is absent; it also produces a strong hemolytic effect.

In addition to the hemolytic action upon blood corpuscles, the saponin masses were identified by their solubility in water, alcohol, phenol, and glacial acetic acid and by their insolubility in ether and chloroform. They also showed characteristic color changes with sulfuric acid, slowly changing from distinctly yellow to pink and reddish violet. The color change to pink or reddish violet could be observed almost immediately, if a mixture of equal parts of sulfuric acid and alcohol, or acetic anhydride followed by sulfuric acid, were used. A green color (not precipitate) was produced by

sulfuric acid containing less than 1 per cent of ferric chloride. If dried plant material is used, 70 or 80 per cent sulfuric acid is preferable to the more concentrated acid, which usually chars or destroys the tissue. The epidermis of the leaves, however, is so strongly suberized that the walls often resist the action of even concentrated sulfuric acid. The stomata imbedded in these epidermis cells at times gave a yellow color, turning to a distinct pink-red. Although this may have been due to sugars or gums which, in the presence of other substances, often give a color similar to that produced by saponins, it is possible also that some of the saponin enters these cells with the cell sap or is at times stored to retain or attract moisture. A 1 per cent potassium permanganate solution was also tried and was instantly decolorized by sections containing saponin. It is questionable, however, whether potassium permanganate which has been used as a reagent for substances of a saponin character is satisfactory for the microchemical detection and location.

The microchemical identification of the saponin by means of the products of hydrolysis has so far not been generally successful, although in sections exposed to the vapor of hydrochloric acid or left immersed for more than a day in 40 per cent sulfuric acid, the formation of crystals has, in a few instances, been observed within the saponin masses, which ordinarily showed no refraction of light.

Isolation of the Saponin.

From 1 to 10 kilos of the finely divided, air-dried rootstocks of *Agave lechuguilla* were extracted several times with hot 95 per cent alcohol, and the red alcoholic solution was evaporated to dryness on the steam bath after adding about 50 gm. of magnesium oxide for each kilo of the agave used. The residue was ground and extracted with hot absolute alcohol. The process was repeated until no more saponin could be obtained. On cooling the alcoholic solutions, a light-colored granular substance separated. This was filtered off by suction and washed once or twice with dry ether. The yield of crude saponin was about 9 per cent. The crude substance was again dissolved in hot absolute alcohol and the saponin allowed to come out by cooling. Repeating the process five times gave an almost white material which contained only a negligible trace of mineral matter.

The saponin remaining in the alcoholic filtrates was obtained by pouring the solutions into equal parts of ether, decanting, re-dissolving the precipitate in hot absolute alcohol, and allowing the substance to come out by cooling. By purifying several times in this way, more pure material was obtained. The same saponin was also isolated in the manner already described from the bases of the leaves, using only the white portion of the base.

The saponin was soluble in water, alcohol, phenol, and glacial acetic acid. Its aqueous solution foamed when shaken. When treated with concentrated sulfuric acid the color change from yellow to purple-red, generally produced by saponins, was obtained. Neutral lead acetate, basic lead acetate, and barium hydroxide did not precipitate it from either the aqueous or alcoholic solution. A 1 per cent solution of the saponin in water did not give a precipitate on adding a 1 per cent alcoholic solution of cholesterol. The saponin was very hygroscopic, and for analysis it was necessary to dry it carefully by gradually heating it to 110°C. in a vacuum oven until the weight was constant.

The following results were obtained on analyzing five preparations:

1.	0.1649 gm. substance:	0.3380 gm. CO ₂	and	0.1179 gm. H ₂ O.
2.	0.1637 " "	0.3359 " "	" "	0.1178 " "
3.	0.1396 " "	0.2857 " "	" "	0.1008 " "
4.	0.2268 " "	0.4646 " "	" "	0.1587 " "
5.	0.0880 " "	0.1910 " "	" "	0.0635 " "

Preparation.....	1	2	3	4	5	Average.
C.....	55.90	55.96	55.82	55.87	56.13	55.93
H.....	8.00	8.05	8.08	7.83	8.08	8.01
O.....	36.10	36.99	36.10	36.30	35.79	36.06

Molecular Weight in Phenol.—0.5341 gm. of substance lowered the freezing point of 27.94 gm. of phenol, 0.229°.

$$M = \frac{7,500 \times 0.5341}{0.229 \times 27.94} = 626. \quad \text{Check 626.}$$

C₂₇H₄₄O₁₂. Calculated. C 57.85, H 7.92, Mol. wt. 560.
Found. " 55.93, " 8.01, " " 626.

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Further analyses carried out¹ with saponin, after further purification, did not yield materially different results:

Average analysis.		
	Before purification.	After purification.
	<i>per cent</i>	<i>per cent</i>
C	55.46	55.32
H	7.62	7.75

*Surface Tension of Saponin.*²—For the surface tension determination, the Morgan drop-weight method was used. At 37°C. the surface tension of a solution of 100 mg. per liter of Locke's³ solution was 59.75 dynes per cm.

Biological Tests.

*Hemolysis of Saponin.*²—For the hemolysis tests, blood was drawn from the heart of a Belgian hare into 0.9 per cent saline solution, to which a little potassium oxalate was added. The blood was then centrifugated and washed with Locke's solution. 2 drops of the residual corpuscles were mixed with 10 cc. of an aqueous saponin solution, 1 in 10,000, and kept at 37°C. Hemolysis took place in about 1 hour.

Toxicological Effect of the Saponin on Fish.

In order to test the effect of the saponin on fish, experiments were made with minnows about 8 to 10 cm. long. The minnows were placed in dishes containing tap water and small quantities of the isolated saponin were added, the highest concentration used being 0.02 per cent. As soon as the saponin had been added the

¹ Analyses made by Mr. C. E. F. Gersdorff, Protein Investigation Laboratory, Bureau of Chemistry.

² Determined by Dr. E. H. Woodward of this Bureau.

³ Locke's solution:

	<i>gm.</i>
NaCl.....	9.2
KCl.....	0.42
CaCl ₂	0.24
HNHCO ₃	0.15
H ₂ O to.....	1 liter

fish became greatly excited and swam about rapidly, but soon calmed down and came to the surface of the water gasping for air. Bleeding in the vicinity of the gills and fins was observed. The fish lost their power of maintaining equilibrium and gradually turned over on their backs. After swimming in this position for sometime they died. The addition of cholesterol delayed and in some instances inhibited fatal action of the saponin (12). In order to compare the toxicity of the agave saponin with that of saponin obtained from other sources, experiments were made under similar conditions with a commercial sample of saponin from *Quillaja saponaria* Merck and a sample of saponin isolated by the authors from California soap-plant, *Chlorogalum pomeridianum* (13). The solution of the agave saponin caused the fish to turn completely over in from 3 to 5 minutes, while the same effect was produced by the quillaja saponin in 15 minutes and by the chlorogalum saponin in 2 hours. The agave saponin was, therefore, the most toxic of the three.

Hydrolysis of the Saponin.

Formation of a Pro-Sapogenin.—50 gm. of saponin were dissolved in 300 cc. of 1 per cent sulfuric acid and the solution was heated on a steam bath for 18 hours. A tan-colored, amorphous pro-sapogenin settled out during the heating. This was thrown down in a compact mass by means of the centrifuge, the supernatant liquid was decanted, and the residue was washed until the washings were no longer acid to litmus. The pro-sapogenin was further hydrolyzed as described later.

Identification of Glucose.—The acid solution decanted from the pro-sapogenin as just described was combined with the wash water, and sulfuric acid was added until the concentration of the acid reached 5 per cent. This mixture was heated on a steam bath for 6 hours, during which time a tarry substance separated. The mother liquor was decanted and neutralized with barium carbonate, and the barium sulfate was filtered off. The filtrate was decolorized with animal charcoal and after filtering was evaporated down, giving a light yellow sirup. A solution of this sirup was strongly dextro-rotatory. The specific rotation of the sugar calculated as glucose was 58.8° to the right at 20°C . using sodium light. The specific rotation of glucose is 53° to the right, while

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the specific rotation of galactose is 81° to the right. The rotation obtained was, therefore, somewhat high for glucose, but this is explained by the fact that the solution contained some galactose. Since mannose was absent and the solution was dextro-rotatory, the formation of phenylosazone (M.P. 205°) and para-bromophenylosazone (M.P. 222°) shows that glucose was one of the hydrolytic products.

Formation of the End-Sapogenin.—When the pro-sapogenin was boiled with 6 per cent sulfuric acid until foaming ceased, the end-sapogenin was obtained in granular form. This was filtered off and was freed from acid by means of water. It was then dissolved in hot dilute alcohol, from which a white crystalline substance separated on cooling. Upon concentration of the mother liquor the same substance formed plates and prisms in addition to needles. The plates were undoubtedly somewhat impure, melting at 180° . The crystal mass was further purified by recrystallization from 95 per cent alcohol. In this manner fine acicular crystals or prisms, melting at 183.5°C ., were obtained. The substance was insoluble in water, but soluble in alcohol, acetone, benzene, and phenol. It was insoluble in dilute acids and alkali, but dissolved easily in concentrated acids. The sapogenin appears to be a very stable compound. When heated at 100°C . for 6 hours in an alcoholic solution of potassium hydroxide, saturated in the cold, it was recovered unaltered. A mixture of potassium dichromate and 50 per cent sulfuric acid also has no action on it.

The analyses of this compound gave the following results:

0.2805 gm. substance:	0.2560 gm. H_2O	and 0.7827 gm. CO_2 .
0.2278 " "	0.2154 " "	0.6362 " "
0.1811 " "	0.1650 " "	0.5041 " "
0.1609 " "	0.1505 " "	0.4487 " "

	Preparation 1.	Check.	Preparation 2.	Check.	Average.
C	76.10	76.10	76.17	76.10	76.14
H	10.21	10.21	10.43	10.58	10.32

Molecular Weight in Phenol.—0.1980 gm. substance lowered the freezing point of 29.30 gm. of phenol 0.206° .

$$M = \frac{7,500 \times 0.1980}{29.35 \times 0.206} = 246. \quad \text{Check } \begin{cases} 245 \\ 246 \\ 245 \end{cases}$$

$C_{15}H_{24}O_2$. Calculated. C 76.27, H 10.17, Mol. wt. 236.
 Found. " 76.14, " 10.32, " " 246.

From its solubility, molecular weight, melting point, and crystallographic and optical properties, the sapogenin appears to be identical with the yucca sapogenin.

*Crystallographic and Optical Properties of Sapogenin from Agave.*⁴

Crystallography.—System, monoclinic; axial ratio $a:b:c = 1.515 : 1 : 2.006$, all ± 0.001 ; axial angle $\beta = 85^\circ 45' \pm 3'$. Forms: c (001), a (100), m (110), d (101), and p (111) developed in the order named. Habit mostly tabular on c and elongated on axis b, thus agreeing with Fedorov's rule as to the relation between axial ratio and habit. Faces somewhat rounded, often slightly concave; signals accordingly not particularly well reflected.

ANGLE TABLE.

No.	Form.	Symbols.		Development.	φ	ρ
		Gdt.	Mill.			
1	c	0	001	Dominant.	90° 00'	4° 15'
2	a	$\infty 0$	100	Prominent.	90 00	90 00
3	m	∞	110	"	33 30	90 00
4	d	10	101	Narrow.	90 00	54 30
5	p	1	111	{ Small.	35 \pm	68 \pm
				{ Calculated.	34 57	67 47

Optical Properties.—Large crystals show parallel extinction and optic axial plane coinciding with plane of symmetry, one optic axis emerging obliquely through base. When crushed yields angular fragments; attacked but slowly by immersion oils; refractive indices (D). $\alpha = 1.535$, $\beta = 1.550$, $\gamma = 1.570$, $\gamma - \alpha = 0.035$, all ± 0.005 ; means are usually shown. Double refraction strong, colors being first to second order on small grains; extinction and elongation indeterminate; class biaxial; 2 E very large; sign +; dispersion distinct.

Isolation of Galactose.—The acid solution obtained from hydrolysis of the pro-sapogenin was neutralized with barium carbonate. After filtering off the barium sulfate, the filtrate was clarified with animal charcoal and evaporated to a sirup. This was dissolved in cold 95 per cent alcohol, and absolute alcohol was added until

⁴ Determined by Dr. E. T. Wherry, Crystallographer, Bureau of Chemistry.

no more gummy precipitate was produced. After standing over night the supernatant liquid was decanted. This was mixed with an equal volume of anhydrous ether and the precipitate was allowed to settle. The mother liquor was decanted and the white residue was crystallized from about 98 per cent alcohol. Small acicular crystals melting at 165°C. were obtained. When mixed with pure galactose, there was no lowering of the melting point. Evaporation of the sugar with nitric acid gave mucic acid. The phenylosazone of the sugar melted at 197°C. and the para-bromophenylylhydrazone melted at 168°C. There is, therefore, no doubt that this sugar was galactose.

SUMMARY.

1. A saponin not hitherto described was isolated from the rootstock with attached roots and short overground axis, and the bases of the leaves of *Agave lechuguilla*. The results of the ultimate analyses, the molecular weight determination in phenol, the nature of the products of hydrolysis of the saponin, and especially the results of the analysis of the sapogenin suggested the formula $C_{27}H_{44}O_{12}$. The saponin was soluble in water, alcohol, and phenol. Lead acetate, lead subacetate, and barium hydroxide did not precipitate it from either the aqueous or alcoholic solutions, nor did cholesterol form an insoluble compound. The aqueous solution containing 100 mg. of saponin per liter hemolyzed rabbit's blood in about 1 hour at 37°C. Its surface tension at 37°C. in Locke's solution was 59.75 dynes per cm.

2. Experiments with fish indicated that the saponin is more toxic than that from the common soapbark, *Quillaja saponaria*, or from the California soap-plant, *Chlorogalum pomeridianum*.

3. The saponin occurs in the cell sap and may be located in the air-dried plant in the fibrovascular bundles or veins of the roots, rootstock, axis, and leaves. The rootstock and roots, apparently containing the largest amounts, yielded in an air-dried state about 9 per cent of crude saponin.

4. The hydrolysis of this saponin yielded glucose and a pro-sapogenin.

5. The hydrolysis of the pro-sapogenin gave galactose and an end-sapogenin.

6. The end-sapogenin, melting at 183.5°C., and to which the formula $C_{15}H_{24}O_2$ is assigned, proved to be identical with a sapogenin previously obtained from a saponin from *Yucca filamentosa*. Its crystallographic optical properties were determined.

BIBLIOGRAPHY.

1. Michotte, F., L'agave culture et exploitation, 1914, 26, 28.
2. Watson, S., *Proc. Am. Acad. Sc.*, 1876, xi, 16. (Reference misquoted in *Kew. Bull.* 23, 1887, 6.)
3. Mexican fibre or ixtle, *Kew Bull.* 23, 1887, 6.
4. Engelmann, G., Notes on agave, *Tr. Acad. Sc. St. Louis*, 1868-77, ix, 306.
5. Baker, J. G., Handbook of the Amaryllideae, 1888, 168.
6. Mulford, A. I., A study of the agaves of the United States, *Mo. Bot. Garden*, 7th Rep., 1896, 76, 77.
7. Rose, J. N., Notes on useful plants of Mexico, *Contrib. from U. S. Nat. Herbarium*, 1897-1901, v, 242.
8. Havard, V., Report on the flora of western and southern Texas, *Proc. U. S. Nat'l. Mus.*, 1885, 518.
9. Dewey, L. H., in von Wiesner, I., and Baer, H., Beiträge zur Kenntnis der Anatomie des Agaveblattes, *Sitzungsber. k. Akad. Wissensch. Math.-naturw. Cl., Wien.*, 1914, cxxiii, 682.
10. Kobert-Rostock, R., Die Saponine, in Abderhalden, E., Handbuch der Biochemischen Arbeitsmethoden, Berlin, 1912, vii, 150, 224.
11. Chernoff, L. H., Viehovever, A., and Johns, C. O., A saponin from *Yucca filamentosa*, *J. Biol. Chem.*, 1916-17, xxviii, 437.
12. Windaus, A., Über die Entgiftung der Saponine durch Cholesterin, *Ber. chem. Ges.*, 1909, xlii, 238.
13. Johns, C. O., and Viehovever, A., The saponins from *Chlorogalum pom-eridianum* and *Agave lechuguilla*, *Science*, 1915, xlii, 72.