

TABLE V.

	I.	II.	III.	IV.	V.
Volume of sample	5.00 cc.	5.00 cc.	5.00 cc.	5.00 cc.	5.00 cc.
Volume diluted to	500.00 cc.	500.00 cc.	500.00 cc.	500.00 cc.	500.00 cc.
Normality of KMnO_4	0.0731	0.0731	0.0692	0.0692	0.0692
Volume of KMnO_4 (A)	11.30	11.32	13.00	13.32	13.35
Volume of KMnO_4 (B)	10.10	10.70	11.19	11.35	11.79
A minus B	1.4	0.62	1.81	1.77	1.76
Tannin equivalents	42.9 mg./cc.	19.0 mg./cc.	52.4 mg./cc.	51.4 mg./cc.	41.4 mg./cc.
Alterations in procedure	Not filtered	Filtered	10 cc. glycerin added	25 cc. glycerin added	100 cc. glycerin added

SUMMARY.

1. Arbutin is not extracted from uva ursi by ethyl acetate, xylol, *n*-butanol or hexane.
2. It was found that the drug, which had been treated in the autoclave previous to percolation, yielded a fluidextract whose precipitate was not only less in quantity, but amorphous rather than crystalline in character. This suggests that enzymes may be the primary cause of precipitation in this preparation.
3. A colorimetric assay for arbutin has been devised which was found to give results comparable to the Zechner process.
4. Filtering a turbid infusion of the crude drug, previous to the determination of the tannin, gives low values of the latter and should be avoided.

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A STUDY OF PRECIPITATION IN FLUIDEXTRACT OF UVA URSI III.*¹

IDENTIFICATION OF THE CRYSTALLINE PRECIPITATE IN FLUIDEXTRACT OF UVA URSI.

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INTRODUCTION.

The manufacture and storage of fluidextract of uva ursi has been a troublesome problem due to the fact that it continues to develop a sediment over a long

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period of time. Scoville (7) suggested that this was due to a hydrolysis of the tannin present. Tisher (11) observed that the precipitate was microcrystalline in character. He studied its physical properties but was unable to identify it. Ball (13) followed up the work of Tisher and showed that precipitation in fluidextract of uva ursi could be changed in character and lessened in amount by subjecting the drug to autoclave heat before extracting it.

The object of this study was that of the identification of the micro-crystals which constitute the larger part of the precipitate in the official fluidextracts of uva ursi. To do this it was necessary to repeat much of the work of Tisher, Ball and others, but the details will be omitted here.

CONSTITUENTS OF UVA URSI.

The glucoside *arbutin* is, perhaps, the most important therapeutic constituent of uva ursi. It yields hydroquinone upon hydrolysis, to which the urinary anti-septic action of uva ursi and its preparations is generally attributed. Arbutin is accompanied, according to Zechner (9), by varying amounts of its methyl derivative.

Urson, more recently named ursolic acid, also occurs in uva ursi. Its chemical nature seems to be uncertain. It has been classed by van der Haar (6) as a phytosterol-like substance with a formula $C_{30}H_{48}O_3$, identical with prunol from the leaves of *Prunus serotina*, and malol from the wax coats of apples.

Ericolin, $C_{34}H_{56}O_{21}$, was isolated by Kawalier in 1852. It is a brownish yellow, sticky resin, of bitter taste, which upon hydrolysis yields glucose and ericinol, $C_{10}H_6O$, a volatile, peculiarly odorous oil.

Other constituents, according to Wehmer (10), which have been isolated from uva ursi are, gallic acid, tannin, quercetin, myricetin, citric acid, malic acid, resin, wax, fat, quinic acid, and formic acid.

Perkin (2) reported the presence of ellagic acid but his results were not confirmed by Keegan (5).

EXPERIMENTAL.

Our first task was that of collecting a quantity of the crystalline precipitate for study. This was done by preparing a number of samples of the official fluidextract in 500- and 1000-cc. amounts. This afforded opportunities to observe the appearance, character, and rate of formation of the precipitate.

It was clearly shown that the crystalline precipitate was not extracted from the drug but formed in the percolate upon standing a few hours.

The precipitate was found to be, for the most part, composed of slender, needle-like, micro-crystals with small bits of plant tissue and coloring matter adhering to them. When dried, after being washed with water, they were of a gray-green appearance.

EXAMINATION OF THE CRYSTALLINE PRECIPITATE.

Ignition.—Upon being ignited the crystals burned with a yellow luminous flame, yielding a very small ash residue, indicating the organic nature of the precipitate.

Calcination.—When the precipitate was subjected to calcination a yellow, crystalline sublimate appeared. Additional heating caused the sublimate to pass off as a yellow, odorless vapor. This observation deserves further study.

Melting Point.—The precipitate was subjected to capillary tube melting point tests. It failed to melt at 360° but darkened to a light brown.

Sodium Analysis.—By means of sodium decomposition and subsequent analysis, sulfur, nitrogen and the halogens were shown to be absent. This was indicative of the organic nature of the precipitate.

Inorganic Analysis.—When treated with concentrated nitric acid a reaction occurred with evolution of brown fumes of NO_2 and a reddish brown liquid was the result. This liquid gave positive tests for the presence of iron, aluminum, potassium and silicates, in small amounts, together with traces of calcium. The acid-soluble ash from the ignition of the precipitate gave tests for the presence of these same elements.

Solubility Tests.—The solubility of the precipitate was tested according to Kamm's (12) classification reagents. The results were as follows:

Insoluble in water, ether and 5% HCl
Partially soluble in KOH 5%, and sulfuric acid
Warm sulfuric acid produced a charring effect

Ferric Chloride Test.—Ferric chloride T.S., when added to the dry precipitate, gave a green coloration which developed into an inky blue upon standing.

CONCLUSIONS.

These tests led to the conclusion that the crystalline precipitate was chiefly organic in character. The ferric chloride test indicated that it was a phenol of some kind.

ATTEMPTS TO PURIFY THE CRYSTALLINE PRECIPITATE.

The recrystallization of a substance from a suitable solvent is often an acceptable means of purification, but in this case the precipitate resisted solution in any of the ordinary solvents. It became dispersed when subjected to hot methyl, ethyl and butyl alcohols but was not dissolved to any extent.

Dilute solution of potassium hydroxide acted as a solvent and upon acidulating the solution with hydrochloric acid a precipitate was produced. From all appearances it was assumed, however, that the original crystals had been decomposed by the alkali and that the precipitate from the acid solution was not a purified product.

THE CRYSTALLINE PRECIPITATE IDENTIFIED AS ELLAGIC ACID.

Perkin (2) reported that ellagic acid was present in *uva ursi*. The descriptions for it, both chemical and physical, suited those of our crystalline precipitate. A preliminary analysis upon the impure precipitate showed a carbon and hydrogen content which approximated that of ellagic acid. The crystalline precipitate also gave the Griessmayer (1) test for ellagic acid.

The purification of ellagic acid, by recrystallizing it from a hot pyridine solution, was reported by Perkin and Nierenstein (3). They also obtained an acetyl derivative of it which gave a melting point of 343–346°.

On the assumption that our crystalline precipitate was essentially ellagic acid, it was purified by repeated recrystallization from boiling pyridine. The purified crystals were washed with ethyl alcohol and dried for twenty-four hours at 120° and subjected to an elementary micro-analysis, the results of which are given in Table I following.

TABLE I.

Weight of Sample.	Weight of Carbon Dioxide.	Weight of Water.	Per Cent Carbon.	Per Cent Hydrogen.
0.018779 Gm.	0.038737 Gm.	0.003902 Gm.	56.25	2.31
0.018913 Gm.	0.038553 Gm.	0.003788 Gm.	55.59	2.23
0.017413 Gm.	0.035477 Gm.	0.003964 Gm.	55.56	2.53

The percentage of carbon in ellagic acid, $\text{C}_{14}\text{H}_6\text{O}_8$, is 55.63 and that of hydrogen 1.99.

From the results of this analysis and the physical characteristics of the crystalline precipitate, which forms in fluidextract of uva ursi, we have concluded that it is identical with ellagic acid.

This assumption is further supported by the fact that we were able to prepare by the method of Perkin and Nierenstein (3), an acetyl derivative of our precipitate which melted at 342–345°.

ELLAGIC ACID AND ELLAGITANNIN.

Ellagitannic acid, from which ellagic acid is produced by hydrolysis, is one of the most widely diffused of all the natural yellow coloring matters. It is often found with gallotannin in nature. Its most common sources are divi divi pods, knopper galls, myrobalans, and other tannin-like substances. The separation of ellagic acid from extracts of these tannin-bearing plant materials has often been observed. The cause of its formation, according to most investigators, is due to the action of a ferment, or enzyme, which hydrolyzes the ellagitannin into ellagic acid and its other components.

ELLAGIC ACID IN UVA URSI.

Perkin (2) reported that ellagic acid existed in uva ursi leaves. Using the method of Nierenstein (4) for the detection of ellagic acid we were unable to detect it, *per se*, in uva ursi. A search of the literature also revealed the fact that ellagic acid, as such, has never been shown to exist, with certainty, in any plant tissue.

SUMMARY.

1. The microcrystalline precipitate occurring in the official Fluidextract of Uva Ursi has been identified as ellagic acid.
2. It has been shown that ellagic acid does not exist in uva ursi but is formed in the fluidextract upon standing.

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The Program of the Scientific Section listed eighty-three papers for the 85th annual meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION, held in New York City.

Dr. Autun Urgoc-Richard Wasicky, *director* of the Pharmacological Institute, Vienna, Austria, was elected *Honorary Member* of the AMERICAN PHARMACEUTICAL ASSOCIATION.
