A PHYTOCHEMICAL STUDY
OF
ARCTOSTAPHYLOS UVA-URSI (Linne)Sprengel

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

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APPROVED Edward Kremers

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Ellagic Acid

Introduction

During the past century Uva Ursi has been one of the common medicinal plants in this country and in Europe, although it appears to have fallen more or less into disuse in recent years. The author first became interested in this plant in 1935-36 while working on the identification of the crystalline component of the precipitate occuring in the Fluidextract of Uva Ursi and a means of preventing the formation of this precipitate. The interest stimulated during this work lead to the undertaking of the present phytochemical investigation of the plant and of the literature concerning its constituents.

Scientific Names and Synonyms for Uva Ursi

C. Clusius, in 1601,[1] listed the plant simply as Uva Ursi. To fit into his binomial system, in 1753, Linne' coined the name, Arbutus Uva-ursi,[2] for what he had previously called Arbutus caulibus procumbentibus, foliis integerrimis. Linne' had used the latter name in his Hortus Cliffortianus,[3] Flora lapponica,[4] Flora suecica,[5] and Materia medica.[6] Royen,[7] and Haller[8] used the descriptive name as used by Linne'. J. Bauhin and Cherler[9] had listed the plant as Radix idaea putata & Uva ursi. Linne', in his Species Plantarum[2] states that Colden listed the plant as Arbutus noveboracensibus Bearberry, but according to a photostatic copy of Colden's original,[10] it is listed as Arbutus Nostratibus Bearberry.

The present name of the plant, Arctostaphylos Uva-ursi, was given to it by Sprengel[11] in 1825, who also listed the descriptive name of Arctostaphylos caule procumbente fruticoso, foliis obovatis integerrimis coriaceis nitidis, floribus fasciculatis, (Arbutus L.).

Post-Linnean names for Arctostaphylos Uva-ursi (Linne) Sprengel, were given by various authors as follows:

Arbutus procumbens, Salisbury,[12] in 1796.

Arbutus buxifolia, Stokes,[13] in 1812.

Arctostaphylos officinalis, Wimmer and Grabowski,[14] in 1827-9.

Arbutus officinalis, Boissier,[15] in 1867.

Common Names

Arctostaphylos Uva-ursi, (Linne), Sprengel, is commonly

known as Bearberry or Bärentraube. Other common German names listed for the plant are: Bärenkraut, Moosbeere, Soltebeere, Achelkraut, Bärentraubenblätter.[17]

Common names used in English speaking countries include: Mountain Box, Red Berry, Upland Cranberry, Bear's Grape, Red-Berried Trailing Arbutus, Whortleberry (generally applied to species of Vaccinium, especially V. Vitis-Idaea, L.), Barren Myrtle, Rockberry, Kinnikinnic, and Uvae Ursi Folia (Br.).[16,18]

Common names in other languages are: Busserole, Raisin d'Ours, Hethe, Universe (Fr.), Uva Ursina (Ital.), and Gayuba (Span.).

Etymology

Arctostaphylos- Millspaugh states that this is derived from the Greek words, ΑΡΚΤΟΣ, arktos, meaning a bear, and σταφυλη', staphyle, meaning a grape or berry because of the rough hair on the fruit.[16]

Uva-ursi- Tschirch states that these names are derived from the Latin, ursus, or Bär (a bear), and uva, or Traube (a grape).[17]

Arbutus- ar= rauh, or herbe (harsh, rough or raw), and butus, βα'τος = Busch (bush or shrub).[17]

Description of the Plant

Arctostaphylos Uva-ursi, (Linne), Sprengel, (Family Ericaceae) is a low, evergreen shrub, with trailing stems, the young branches of which rise obliquely upward for a few inches.

Millspaugh gives the following description: [16]

"This peculiar boreal shrub is seldom erect except that it throws its shoots upward for from 3 to 8 inches. Stems numerous, depressed or trailing; branches various, the sterile from 2 to 3 feet long and compactly leafy, the fertile shorter; bark mahogany color, scaling off in irregular patches; roots thick, ligneous, and creeping. Leaves alternate, coriaceous, thick, shining, and evergreen, turning mahogany color when aged, those of the erect branchlets more or less vertical, all oblong, spatulate, entire, retuse, and tapering to a short-petioled base. Inflorescence in few-flowered, terminal clusters or racemes; bracts and bracteoles persistent, finally becoming rigid; flowers pale, rose colored, drooping. Calyx reddish, persistent, free from the ovary; lobes 5, roundish. Corolla urceolate, pellucid at the base, deciduous; tube inflated, hairy inside, hypogynous; lobes 5, short, acute recurved. Stamens 10, included; anthers large, upright, intorse, the cells opening by terminal pores and appendaged upon the dorsal surface by 2 reflexed awns. Ovary 4 to 10 celled; ovules solitary in each cell. Fruit a glabrous, depressed-globose berry or drupe, about the size of a pea; pulp mealy and insipid; nutlets 5, when the fruit is baccate, or united firmly into a 5-several celled stone when drupaceous; whether distinct or coherent, the nutlets are bony and 1-nerved upon the dorsal surface."

Habitat

The Bearberry is indigenous to the northern latitudes and high mountains of Europe, Asia, and North America. In North America it extends from the Hudson Bay as far southward as New Jersey and Pennsylvania, where, in certain localities it grows in abundance. It extends westward in this country through Michigan, and Wisconsin and into northern California. In Europe it extends northward from northwestern Ireland, Yorkshire, England, and central Russia, and extends southward into the Tirolean Alps and the mountains of Spain, where it grows abundantly as a commercial source. In Asia it extends northward from lower Siberia and Kamtschatka; its northerly range includes Iceland and Greenland. [16,17,18]

The Bearberry is a hardy shrub, preferring barren soil among rocks and flourishing on graveled hills and high sandy plains. It flowers in May and the leaves for use in medicine should be gathered in autumn.

The chief adulterant for the Uva Ursi of commerce is the inert leaves of Vaccinium Vitis-Idaea, Linne, (Cowberry or Mountain Cranberry); these may be distinguished by being obovate, having resolute margins which are sometimes slightly toothed, and the presence of fine black dots or bristly points upon their lower surface. Other less common adulterants are Vaccinium Myrtillus, Linne, Chimaphila umbellate, (Linne) Nuttall, and Arctostaphylos glauca, Lindley. The latter, commonly called Manzanita, is a small shrub indigenous to California where it grows in dry and rocky places on the west slopes of the mountain ranges.

History

The Bärentraube appears to have been used in northern European medicine for a long time. It appeared in the medical books of England in the 13th century, in the Meddygon Myddfai, and was used by the "physicians of Myddfai".[17] Clusius described the plant in 1601 as the Αρκτου σταφυλη of Galen, useful as a hemostatic.[16] Its use did not spread into middle Europe until sometime later.[17] Tschirch states that in C. Bauhin's Pinax the plant was listed under the name, Vitis Idaea folliis carnosis et veluti punctatis sive Idaea radix, and that it was first found to be used in Spanish, Italian, and French medicine about

the middle of the 18th century as an astringent and in nephritic disorders. In Germany it became especially well known through its use by DeHaen in Wien (Ratio medendi, 1758), by Gerhard in Berlin (Die Bärentraube chymisch-medizinisch betrachtet, 1763), and by Murray in Gottingen (Commemtatio de Arbuto uva ursi, 1765). In Spain it was used by Girardi (Dissertacion sobre la passion nephritica y su verdadero specifica la Uva-ursi o Gayubas, Madrid, 1763).[17] It was admitted to the London Pharmacopoeia in 1763.

In America the Aborigines smoked the dried leaves with tobacco, making a mixture called Sagack-homi in Canada, and Kinikinik among the western tribes; this was the Larb of the western hunters.[16]

Medicinal Uses

As has been stated, Uva Ursi was used by the ancients as an astringent. After the middle of the 18th Century it came into more or less general use as an astringent tonic and diuretic in various disorders, particularly in dysuria, chronic vesical catarrh, cystitis, and kindred afflictions, as well as in irritations of the genital tract, such as gleet, gonorrhoea, leucorrhoea, blenorrhoea, etc., Millspaugh[16] states that a Dr. Bourin, of Oxford, recommended it highly in phthisis, but it only abated the fever from reduction of the heart's action.

Uva Ursi has never received the complete support of the medical profession as a genito-urinary antiseptic. Its value is claimed by some to be due to arbutin, the chief constituent,

which is hydrolysed in the body to hydroquinone and glucose, the former being the antiseptic agent.[19] Lewin and Schmiz[20] recommended the substitution of arbutin for ordinary preparations of Uva Ursi in medicinal use, while Paschkis[21] obtained no good results from the use of arbutin in cystitis and gonorrhoea, but found the ailments inproved with the use of Uva Ursi leaves. He attributed the beneficial action to the tannin present in the leaves.

Gauchet[22] considered Uva Ursi to be of advantage in certain cases of labor, and it received some attention as a uterine excitant, useful in prolonged parturition from atony. It was claimed that the contractions resulting from its use were more prolonged, while less painful and less dangerous to the child.

Uva Ursi was admitted to the first edition of the U. S. Pharmacopoeia and has appeared in every revision of that work through the 10th revision. It was deleted from the 11th revision and taken up by the National Formulary VI. A review of the opinions of various authors[23] concerning the value and use of Uva Ursi preparations has shown it to be not highly regarded among the medical profession and in recent years it has found little reception in this country. In European medicine, however, the drug seems to have retained its popularity more favorably in recent years. Werler,[24] in 1900, recommended a combination of salol and Uva Ursi in pill form as a disinfectant, astringent, and tonic in diseases of the bladder and urinary organs, while Polland,[25] as recently as 1927, recommended Uvalsat, a dialysate of Uva Ursi, as a genito-urinary antiseptic. Other

evidences of its use in European medicine are found in the literature in various forms of medication.[26,27]

The various modes of administration of Uva Ursi in medicine have included the powdered leaves, dried extract, syrup, infusion, decoction, fluidextract, pill, and dialysate. Of these the fluidextract and infusion have been most widely used. Because of the formation of hydroquinone in the body the urine often assumes a green color from the use of arbutin or Uva Ursi.

Chronological Survey of the Constituents

The report of Hughes,[28] in 1847, is one of the earliest recorded investigations of the constituents of Uva Ursi. This author reported the leaves to contain tannin, gallic acid, gum-resin, bitter extractive, volatile and fixed oils, lignin, and a crystalline principle obtained from the aqueous extract, to which he gave the name, "Ursin", since it embodied the diuretic power of the leaves.

Kawalier,[29] in 1852, reported the isolation of arbutin, which when treated with emulsin yielded grape sugar and another ether-soluble compound which he called "Arctuvin". He also reported the presence of gallic acid, tannic acid, wax, fat, chlorophyll, plant fiber, a resin-like material, and a substance, "Ericolin", which upon decomposition with dilute acids yielded a volatile oil, "Ericinol", which easily took up oxygen from the air.

H. Trommsdorff,[30] in 1854, reported the isolation of a

new crystalline substance from both the alcoholic and the ether extract of the leaves. He assigned the name, urson (ursone), to this principle, whose presence was later confirmed by Gintl,[31] Nooyen,[32] and others.

While investigating the products of the hydrolysis of arbutin in 1858, Strecker[33] found hydroquinone as a cleavage product and asserted this to be the "Arctuvin" of Kawalier. Hlasiwetz and Habermann,[34] however, in 1875, found that the arbutin of Uva Ursi did not yield pure hydroquinone but a mixture of hydroquinone and methylhydroquinone, indicating, although they apparently did not interpret it as such, that methylarbutin as well as arbutin was present in the leaves. Their results in this respect were confirmed by Schiff,[35] in 1882.

It remained for Jungmann,[36] in 1871, to show that the "Ursin", isolated by Hughes in 1847, was identical with the arbutin of Kawalier. This author also reported the isolation of a resinous body, Ericolin, which was glucosidal in nature, being cleaved by dilute acids into grape sugar and Ericinol, an odorous substance having the character of a volatile oil. Besides these he reported the presence of tannic acid, gallic acid, urson, malic acid, and a small quantity of a volatile oil.

DeGraffe,[37] in 1896, published the results of her investigation of the tannin of Uva Ursi, from which she concluded that it was of the gallotannin type.

Perkin,[38] in 1898, reported the isolation of a yellow coloring matter which was very similar to quercetin. He also

detected the presence of ellagic acid and accordingly stated ellagitannin was present along with gallotannin. He was able to show in 1900 that the coloring matter consisted of ellagic acid, quercetin, and a small amount of another substance which was probably myricetin.[39] The existence of ellagic acid as reported by Perkin was questioned by Keegan, in 1913, who was unable to find either gallic acid or ellagic acid in the plant.[40]

The isolation of a flavonol glycoside, isoquercitrin, was reported in 1935 by Nakamura and his coworkers.[41]

In summary, the following constituents of Uva Ursi leaves have been reported: arbutin, methylarbutin, ursone, ericolin, tannin, gallic acid, ellagic acid, quercetin, myricetin, malic acid, and isoquercitrin.

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Experimental Part

The crude drug used in this work was in the form of a No. 20 powder of Uva Ursi leaves which was generously donated by the Eli Lilly Company, of Indianapolis, Indiana.

Extraction with Ether

Seven thousand grams of the crude drug were packed in a Lloyd extractor and exhausted by continuous percolation with ether until neither any more color nor solid material were extracted. This required about seven days of continuous extraction. The entire ether extract measured approximately 12 liters.

Soon after the extraction had been started a deposit appeared in the bottom of the extractor and this increased in amount during the remainder of the extraction period. After the extraction was complete, the entire ether extract was filtered and the liquid extract separated from this deposit (A).

The filtrate was concentrated to 3000 cc by distillation of the ether. To this concentrate petroleum ether was added with the result that a large amount of precipitate formed. Petroleum ether was added until no further precipitation occurred. This required about 3000 cc of petroleum ether. This precipitate was collected by filtration (B).

The filtrate was again concentrated to 3000 cc, during which time additional material separated, which was collected by filtration (C).

The mother-liquor was then allowed to evaporate spontaneously, leaving a soft, semi-solid residue (D).

In this manner the entire ether extract was separated into the following fractions:

A). The deposit separating from the ether extract during the percolation.

B). The precipitate obtained by the addition of petroleum ether to the concentrated ether extract.

C). The precipitate obtained by concentration of the ether-petroleum ether mother-liquor.

D). The residue remaining from the concentration to dryness of the ether-petroleum ether mother-liquor.

A). The Deposit Separating from the Ether Extract during the Percolation.

Isolation and Purification of Ursolic Acid.

This material when dried was greenish-yellow in color and weighed 160 grams. It was assumed to consist largely of crude ursolic acid. The purification of 42 grams of this material was undertaken after the manner of Sando* in the following way:

The 42 grams were dissolved with stirring in 750 cc of boiling alcohol containing 1% of sodium hydroxide. During this treatment a considerable quantity of brown colored insoluble material settled to the bottom of the beaker in a sticky mass. The solution was filtered and this insoluble mass collected from the bottom of the beaker. Upon cooling it became hard and brittle. It weighed about 10 grams. (A1).

To the filtered solution there was added an equal quantity of hot water. Upon evaporation of the alcohol a semi-crystalline gelatinous material (sodium ursolate) was deposited, which increased in amount on cooling. This was greenish-white in appearance and mixed throughout it were solid particles of a

* J.Biol.Chem. 90,477,1931.

yellow colored material, too finely dispersed, however, to allow a mechanical separation. The entire mass was collected by suction filtration and the mother-liquor reserved for concentration.

The crude sodium ursolate on the filter was again dissolved in 750 cc of the same solvent as before, filtered, and an equal quantity of hot water added to the filtrate. Upon evaporation of the alcohol a quantity of yellow-brown oily particles ~~were~~ Was found floating on the surface of the hot aqueous solution. These were collected by means of a spatula and upon cooling they solidified and became deep yellow in color. These were presumably the same as the solid yellow particles noted above in the first crystallisation. The total quantity of yellow material collected in this way was only a few grams. (A2).

Upon cooling, the hot aqueous solution of sodium ursolate again deposited a semi-crystalline mass. This was collected on a filter and was then treated with 500 cc of cold alcoholic 1% sodium hydroxide. Upon stirring for a short time most of the material dissolved. A small amount of it remained undissolved along with some more of the yellow material noted above. (A2). The mixture was filtered while in this state. The yellow material was recovered from the residue on the filter by treating it with hot petroleum ether, filtering, and evaporating the filtrate. This additional yellow material was added to (A2) above.

The alcoholic filtrate containing the sodium ursolate was again treated with hot water and the alcohol allowed to

evaporate. This procedure was repeated four times in all, after which the sodium ursolate was finally obtained as a white crystalline powder. The mother liquors from all of the crystallisations were reserved for concentration.

Ten grams of the sodium ursolate obtained in this manner were dissolved in 200 cc of hot alcohol, filtered, and to the filtrate was added dilute hydrochloric acid, slowly and with stirring, until the solution became acid in reaction. At this point ursolic acid was precipitated as a gelatinous mass. This was collected on a suction filter, washed with alcohol, and dried on a porous plate. The dry product was then dissolved in 600 cc of hot alcohol, filtered, and the filtrate allowed to cool in a refrigerator. Ursolic acid crystallised in the form of long, slender, glistening needles, pure white in color. This first fraction of the acid was collected and when dried it melted at 278-9° (uncorr.).

The mother-liquor was allowed to concentrate to one-half its volume and then cooled in an ice bath. The second fraction of the acid crystallised out and when dried it melted at 276-7° (uncorr.).

Alternate Method of Treating the Crude Ursolic Acid (A)- The observation having previously been made that the yellow solid material above (A2) was soluble in petroleum ether, it was thought possible to extract this material from (A) by this means, ursolic acid itself being insoluble in petroleum ether. Upon evaporation of the extract, however, it was seen that none or very little of the yellow material had been thus taken out.

The residue from this extraction was green in color.

The residue (A) after extraction with petroleum ether was next extracted with hot water with the purpose in mind of obtaining the dark brown substance (A1) noted above. The aqueous extract upon evaporation left a large quantity of impure tan colored crystalline material which was suspected of being the same as (A1) above.

The residue (A) after extraction with hot water was of the same color and appearance as before this treatment.

(A1). The Portion of Crude Ursolic Acid (A) Insoluble in Alcoholic Sodium Hydroxide.- This 10 grams of material was treated with hot water and it was found that it could be separated in this manner into a water-soluble and a water-insoluble portion.

The water-insoluble portion, when allowed to dry, was light green in color and appeared to be crude ursolic acid which had remained undissolved in the original treatment with alcoholic alkali. It gave the Liebermann-Burchard color test similar to ursolic acid. Upon purification of this portion by the method used above for ursolic acid, it yielded an additional amount of the acid.

Evaporation of the filtrate containing the water-soluble portion left a black, sticky residue which became hard and brittle when dry.

(A2). The Yellow Material Separating from the hot Aqueous Solution of Crude Sodium Ursolate.- This material was of a fat-like consistency and melted at about 60°.

It was soluble in ether, petroleum ether, and ethyl acetate. The quantity of this material collected was not large enough to allow any further characterisation and it was reserved for further study until such time when more of it could be collected.

(A3). The Water-Soluble Portion of Crude Ursolic Acid (A)- This material, left upon evaporation of the water, was light tan in color and crystalline in appearance. It burned with a yellow luminous flame, leaving a small amount of black residue. The crude material melted partially at 108° and completely at 155°, indicating it to be a mixture. Solubility tests showed it to be soluble in water, insoluble in ether, soluble in 5% hydrochloric acid, soluble with darkening in 5% potassium hydroxide, and soluble with slight charring in concentrated sulfuric acid. Tests for the elements showed it to contain no sulfur, nitrogen, or halogen.

An aqueous or alcoholic solution of the material gave a green color with ferric chloride solution and a positive Jungmann test for arbutin. The Schotten-Baumann reaction gave a white crystalline derivative melting at 170°. Refluxing with acetic anhydride for three hours gave a white crystalline derivative melting at 143-5.5°. The acetate of arbutin melts at 144-5°.

An aqueous solution of the material gave a negative test for reducing sugars. After heating a portion of the aqueous solution with dilute hydrochloric acid for a short time it reduced Fehling's solution readily, indicating the

presence of a glucoside.

The entire amount of the material was treated with 200 cc of an alcohol-ether solution (1:1). This separated the material into two portions, one soluble in this solvent, the other insoluble in it.

The insoluble portion was almost white in appearance and bitter in taste. It melted at 196-7°, gave a bluish-green color with ferric chloride solution, and a positive Jungmann test for arbutin. It was then dissolved in 30 cc of water and refluxed with 5 cc of 10% sulfuric acid for 1/2 hour. The hydrolysate was shaken out with ether and the ether upon evaporation left behind a considerable quantity of white crystals, melting at 170-2°, which proved to be hydroquinone. These results identified this portion of the mixture as arbutin.

(B). The Precipitate Obtained from the Concentrated Ether Extract by Addition of Petroleum Ether.

This material, representing a part of the petroleum ether insoluble portion of the ether extract, weighed 184 grams and when dried and reduced to a powder it was dark green in appearance.

Treatment With Sodium Carbonate - Isolation of Hydroquinone and Gallic Acid.

The 184 grams of material were warmed with 100 grams of sodium carbonate in 200 cc of water for 1/2 hour, the mixture filtered, and the residue washed with water.

(B1). The Sodium Carbonate-Soluble Constituents.- The filtrate, dark greenish-brown in appearance, was concentrated to about 150 cc and shaken out repeatedly with ether. Upon evaporation the ether left a considerable quantity of light tan colored crystalline material. (B1a.).

The filtrate, after washing with ether, was acidified with hydrochloric acid and again shaken out repeatedly with ether. Upon evaporation the ether left a considerable quantity of dark brown colored material having an impure crystalline appearance. (B1b.).

The filtrate remaining from this washing with ether was now light brown in color. It was evaporated to dryness and the residue of impure sodium chloride left was extracted with absolute alcohol. The alcohol upon evaporation left a black, brittle residue. (B1c.).

(B1a.). This crude substance was of a light tan

crystalline appearance, odorless, and burned with a luminous flame, leaving no residue. It melted at 161-70°. It was soluble in water, ether, and in 5% potassium hydroxide with a darkening of color. It gave no color with ferric chloride solution. By the Schotten-Baumann reaction it gave a light tan colored crystalline benzoate melting at 199-200°. Acetylation with acetic anhydride gave an acetate, white in color and melting at 122-3°. These results served to identify the compound as hydroquinone.

(Elb.). This material was dark brown in color and sticky in consistency. It left a white ash upon burning. The crude material showed sintering between 150-200° and melted finally at 225°. It was partially soluble in water, giving an acid reaction in solution, and soluble with darkening in 5% potassium hydroxide. It gave a bluish-green color with ferric chloride solution. By the Schotten-Baumann reaction it yielded a benzoate melting at 200°; it also gave an acetate melting at 120-1°. These results showed hydroquinone to be one of the constituents of this mixture.

The entire amount of crude material was dissolved in 5% potassium hydroxide and carbon dioxide was passed through this solution for 12 hours, with the result that a small amount of glistening black material precipitated. This was removed by filtration, the filtrate acidified, and after standing in the refrigerator overnight there had crystallised a tan colored substance melting at 237-40°. Methylation of this substance with dimethyl sulfate yielded a crystalline derivative, melting

at 165-8°. Trimethyl gallic acid melts at 169-70°.

From these results substance (B1b.) was shown to be a mixture of hydroquinone with gallic acid.

A Morpholine Derivative of Gallic Acid.

Five grams of gallic acid were dissolved in 50 cc of alcohol, an equivalent amount of morpholine added, and the solution refluxed gently on the steam bath for 20 minutes. When it was allowed to cool a light green syrupy residue settled to the bottom. The supernatant liquid was decanted and placed in a refrigerator, where, after standing for two days crystals began to form in the bottom of the liquid. These crystals increased in size over a period of two days. When collected and dried they were light tan in color and melted at 185°, with decomposition.

The light green residue collected above was dissolved in hot alcohol, giving a light brown colored solution. Upon cooling a brown syrupy residue settled out, which, upon standing for a few hours, crystallised to a light tan colored mass. When collected and dried these crystals melted at 180°, with decomposition. When recrystallised from alcohol with the aid of animal charcoal the crystalline product melted at 185-7°, with decomposition.

(B1c.). The black, brittle mass left upon the evaporation of the alcoholic extract has not been further investigated.

Treatment with Sodium Hydroxide.

The residue (B) from the sodium carbonate treatment above, which still retained most of its green color, was allowed

to dry and was then warmed with 200 cc of a 1% solution of sodium hydroxide for 1/2 hour, the mixture filtered, and the residue washed with water.

(B2). The Sodium Hydroxide-Soluble Constituents.- The filtrate, dark brown in color, was concentrated to about 150 cc and shaken out repeatedly with ether. Upon evaporation the ether left a small quantity of a light tan colored substance which did not show any crystalline structure. (B2a).

The filtrate, after washing with ether, was acidified with hydrochloric acid and again shaken out repeatedly with ether. Upon evaporation the ether left a small amount of dark greenish-brown, sticky residue. (B2b).

The filtrate remaining from this washing with ether was then evaporated to dryness and the residue of impure sodium chloride left was extracted with absolute alcohol. The alcohol upon evaporation left a small amount of a dark brown brittle residue. (B2c).

(B2a). This substance, weighing about 2 grams, consisted of a light tan powder, non-crystalline in appearance, and melting between 180-200°. It was insbluble in water, dilute acid, and dilute alkali, and dissolved in concentrated sulfuric acid with charring. When tested for the elements it showed the absence of sulfur, nitrogen, and halogens. In alcoholic solution it gave no color with ferric chloride solution. The amount of this substance obtained was too small to allow any further characterisation.

(B2b). and (B2c). These residues have not been further

investigated.

Residue (B) From the Sodium Hydroxide Treatment.-

This residue when collected on the filter and allowed to dry constituted most of the bulk of the original material started with an was now of a light green color. Since this residue gave a color reaction with the Liebermann-Burchard test it was suspected that it contained more ursolic acid. Accordingly, 60 grams of the residue were dissolved in an excess of 1% alcoholic sodium hydroxide, filtered, and an equal quantity of hot water added to the filtrate. When this solution was allowed to cool there separated to the bottom of the beaker an appreciable quantity of a dark yellow colored, semi-solid residue, (B3). This was collected by pouring off the supernatant liquid ~~above it~~. The alcohol was allowed to evaporate from this liquid as in the purification of ursolic acid under (A) and when the aqueous solution left behind was allowed to cool a gelatinous mass, presumably crude sodium ursolate, settled out of it. This gelatinous mass was almost white in color and contaminated with green coloring matter. The mother-liquor was dark green.

Since the sodium salts of organic acids are insoluble in ether and other organic solvents, it was thought possible to remove the green coloring matter from this crude sodium ursolate and the mother-liquor by extraction with an imiscible solvent. The gelatinous mass collected above was allowed to dry and then one portion of it was extracted with ether and another portion extracted with petroleum ether. However, both the ether and

petroleum ether extracts were colored, not green, but a deep yellow. From the petroleum ether extract there settled out upon standing a yellowish-white colored, solid extractive, (B4). The crude sodium ursolate upon further purification was finally obtained as a white crystalline substance which upon acidification yielded more ursolic acid.

(B3). <u>The Yellow, Semi-solid Residue Obtained From the Alcoholic Solution of Crude Sodium Ursolate.</u>

This residue was allowed to dry on a porous plate. When completely dry it was deep yellow in color and could be reduced to a powder by trituration. Its melting point was indefinite, partially at 70° and completely at 135-60°, indicating the substance to be a mixture. It was soluble in ether, acetone, chloroform, and alcohol, and insoluble in water, acid and alkali, and in petroleum ether. Attempts to obtain a crystalline product from this mixture by crystallising from various solvents were not successful. This material was reserved until more of it could be collected for a more detailed characterisation.

(B4). The solid extractive material settling out of the petroleum ether extract of the crude sodium ursolate. Since this material was found to be the same as that isolated at the same point in the treatment of the residue (C) on page 29, it will be taken up there.

(C). The Precipitate Obtained by Concentration of the Mother Liquor After Addition of Petroleum Ether to the Ether Extract.

This material, representing another part of the petroleum ether-insoluble portion of the ether extract, was dark green in appearance and weighed 80 grams.

Treatment With Sodium Carbonate- Isolation of Hydroquinone and An Unidentified Substance.

This 80 grams of material was treated with sodium carbonate solution in a similar manner as Precipitate (B) was treated. This resulted in the separation of the following sodium carbonate-soluble constituents:

(C1a). A tan colored crystalline substance obtained from the ether washings of the sodium carbonate filtrate:- This substance was small in quantity and melted at 169-70°. It was partially soluble in water, insoluble in 5% hydrochloric acid, and soluble in 5% potassium hydroxide. Treatment with hot water separated the material into a water-soluble and a water-insoluble portion. The water-soluble portion melted at 165-72°, gave a benzoate, M. P. 198-200°, and an acetate, M. P. 121-2°, showing it to be hydroquinone. The water-insoluble portion melted at about 160°, was light brown in color, was also insoluble in dilute acid and dilute alkali, and gave no color with ferric chloride solution. Tests for aldehydes, ketones, and alcohols on this portion were negative.

(C1b). A dark brown colored crystalline material obtained from the ether washings of the sodium carbonate filtrate

after acidification with hydrochloric acid:- This substance melted at 171°, was soluble in water, insoluble in dilute acid, but soluble in dilute alkali with darkening in color. It gave a fleeting color with ferric chloride solution, and yielded a benzoate, M. P. 198-200°, showing it to be hydroquinone.

(C1c). The acidified sodium carbonate filtrate was evaporated to dryness and the residue extracted with absolute alcohol. The alcohol upon evaporation left a small amount of a dark brown, brittle residue which was not further investigated.

Treatment With Sodium Hydroxide - Isolation of An Unidentified Substance.

The residue (C) from the sodium carbonate treatment above was allowed to dry and then treated with a 1% sodium hydroxide solution in a manner similar to the treatment of Precipitate (B) above. This resulted in the separation of the following sodium hydroxide-soluble constituents:

(C2a). A light tan colored, non-crystalline powder obtained from the ether washings of the sodium hydroxide filtrate:- This substance melted at 165-9°, was insoluble in water, dilute acid, or dilute alkali. It contained no sulfur, nitrogen, or halogen, gave no color with ferric chloride solution, and gave a negative test for glucosides. In an attempt to crystallise the material it was dissolved in hot alcohol, refluxed with charcoal, and filtered. Nothing crystalline could be obtained from the filtrate and the residue then melted at 195°. Tests for aldehydes and ketones were negative. Refluxing a small amount of the material with acetic

anhydride for several hours had no effect on it, the original, non-crystalline material being obtained again.

(C2b). A dark brown, sticky, resin-like material obtained from the ether washings of the sodium hydroxide filtrate after acidification with hydrochloric acid:- The amount of this material was too small for further investigation.

(C2c). The acidified sodium hydroxide filtrate was evaporated to dryness and the residue extracted with absolute alcohol. The alcohol upon evaporation left a small amount of black, brittle residue which has not been further investigated.

Residue (C) From the Sodium Hydroxide Treatment.

This residue when collected on the filter and allowed to dry constituted most of the bulk of the original material ~~started with~~ but was much lighter green in color.

Extraction of Residue (C) With Petroleum Ether-Isolation of a High Molecular Weight Alcohol.

Since this residue also gave a color reaction with the Liebermann-Burchard test the presence of more ursolic acid was suspected. Accordingly, this residue was dissolved in an excess of hot 1% alcoholic sodium hydroxide, the solution filtered, and an equal quantity of hot water added to the filtrate. When this solution was allowed to cool in an ice bath, there separated out a large amount of a greenish-yellow colored, granular residue. This was collected on a funnel, and the mother-liquor remaining behind was evaporated. It left a very small amount of residue, showing that almost the entire amount of the original residue

(C) had been precipitated from the alkaline alcoholic solution by the addition of water. The residue on the funnel was allowed to dry and then it was extracted in a Soxhlet type extractor with succesive portions of petroleum ether. The petroleum ether soon became saturated with extractive material which separated out in the bottom of the flask. The extraction flask was changed and new solvent added from time to time so that three fractions of the petroleum ether extract were collected. These fractions showed the following properties:

1st fraction- A light yellow colored extractive material settled out of the flask leaving a deep yellow colored liquid above it. The solid extractive melted at 197-205°.

2nd fraction- A light yellow colored extractive material settled out leaving a pale yellow colored liquid above. The solid extractive melted at 203-9°.

3rd fraction- Very pale yellow colored extractive settled out leaving a pale yellow liquid above. Solid extractive melted at 205-7°.

The solid extractive material from each fraction was collected by filtration and allowed to dry on porous plates. That from the second fraction was almost pure white in appearance. It was found to be insoluble in water, dilute acid and dilute alkali. Concentrated sulfuric acid dissolved it with a slight amount of charring. It was soluble in ether, methyl and ethyl alcohol, acetone, hot petroleum ether, and hot heptane. From the latter two solvents it separated in an amorphous form on cooling, but from hot ethyl alcohol, 75%

alcohol, methyl alcohol, and acetone it crystallised on cooling in the form of short, glistening needles.

The entire amount of material from the second fraction was then recrystallised from 75% alcohol with the aid of animal charcoal and was obtained in the form of white crystals , melting at 209-12°. These upon recrystallisation from a mixture of alcohol and acetone gave a still purer product which melted at 216-18° (uncorr.) to a clear colorless liquid.

The solid extractive material from the first and third fractions was then crystallised from an alcohol-acetone solution with the aid of charcoal and it was obtained in pure white crystalline form with a melting point in each case of 217-8°. This showed that the crystalline material was the same from all of the fractions.

Properties of the crystalline compound.- An elementary analysis showed the absence of sulfur, nitrogen, and halogens. Tests for unsaturation with permanganate and bromine were negative. It gave a negative test for aldehydes and ketones and gave no color with ferric chloride solution. With acetyl chloride the compound dissolved and showed some heat of reaction. With the Liebermann-Burchard test it gave a definite color reaction but one which was not a sterol color test.

Optical Rotation.- A 0.5 gram sample was dissolved in 100 cc of acetone and showed a specific rotation of

$$[\alpha]_{10}^{D} = +12.25^{\circ}$$

Preparation of an Acetate.- The behavior with acetyl chloride having indicated the presence of an alcohol group, it

was desirable to prepare an acetate. Two grams of the compound were refluxed with an excess of acetic anhydride for two hours and the reaction product then poured into water. After decomposition of the excess acetic anhydride there remained a light brown colored, thick syrupy residue. This was very difficult to crystallise but it was finally crystallised from a mixture of alcohol and acetone and was obtained from this solvent in the form of a white, needle-like crystalline compound which melted at 128-9°. (uncorr.).

The saponification equivalent of this acetate was obtained as follows:

Weight of sample	0.50000	0.50000
0.5046 N KOH used to saponify	25.00	25.00
0.9704 N HCl used to backtitrate	10.86	11.00
Saponification Equivalent	237.4	257.6

Assuming the presence of one hydroxyl group in the molecule the molecular weight of the acetate would be the same as the saponification equivalent. If it is assumed that two hydroxyl groups are present the molecular weight of the acetate would be twice the value obtained above.

The alcohol, regenerated by the saponification of the acetate, was recrystallised from an alcohol-acetone solution with the aid of charcoal and then had a melting point of 219-222° (uncorr.).

A combustion analysis of this purified product yielded average values of C= 79.8%; H= 11.5%. These values, however, represent approximations only, because the combustion apparatus

used could not be depended upon to give precise results.

Using these approximate carbon and hydrogen values along with the melting points and physical properties of the alcohol and its acetate, a search was made of the literature in an attempt to locate the unknown alcohol among similar products previously isolated. Its identity with any known product could not be established. This search indicated that the unknown alcohol is very probably a new representative of a group of polycyclic alcohols, commonly referred to as "triterpene alcohols". A number of these have been isolated, either as such from natural sources or by the decarboxylation of the corresponding "triterpene acid". Those more commonly known include the Amyrins, $C_{30}H_{49}OH$, Lupeol, $C_{31}H_{49}OH$, Brein, $C_{30}H_{48}(OH)_2$, Hederagenol, $C_{31}H_{50}(OH)_2$, Oleanol, $C_{30}H_{49}OH$, and others. It may be seen that they represent both mono- and di-hydroxy compounds.

Although the high molecular weight alcohol isolated from Uva Ursi has not been identified, the evidence indicates that it will be found to be a compound similar to the examples cited above. This prediction is strengthened by the fact that ursolic acid, a "triterpene acid", exists in the plant and it is not inconceivable that upon decarboxylation ursolic acid would yield such an alcohol. More precise combustion analysis and structural work on the unknown compound must be done to support this assumption. Suffice it to say at present, the compound could not be identified as one which has been previously isolated from a natural source.

(D). The Residue Obtained From the Concentration of the Mother Liquor Remaining From the Precipitation of the Concentrated Ether Extract With Petroleum Ether.

This dark green, soft, sticky residue, represented the petroleum ether-soluble portion of the ether extract.

Treatment With Sodium Carbonate.

This treatment, carried out as with Precipitates (B) and (C) above, yielded only traces of materials.

Treatment With Sodium Hydroxide.

This treatment, carried out as with Precipitates (B) and (C) above, yielded nothing

Residue (D) From the Sodium Hydroxide Treatment:-

Twenty five grams of this residue were refluxed for one hour with an excess of alcoholic potassium hydroxide in order to saponify it. Upon cooling there separated out of the saponified products a considerable quantity of tan colored crystalline material which proved on examination to be an inorganic potassium salt. Its presence was explained by the fact that sodium carbonate and sodium hydroxide had been left behind in the treatment above of the original residue.

Solvent Experiments on Residue (D).

Small portions of this dark green, sticky residue were shaken with 10 cc each of a variety of cold organic solvents in test tubes. These solvents, with the results observed, are arranged in the following table:

SOLVENT	EFFECT
petroleum ether	partial solution, with green color in upper layer.

SOLVENT	EFFECT
n-heptane	same as petroleum ether.
benzene	dissolves some green color; residue lighter in color.
xylene	almost complete solution.
chloroform	almost complete solution; some solid material separates to top on standing.
ethylene dichloride	same as chloroform.
carbon tetrachloride	partial solution; residue in fine suspension.
methyl alcohol	dissolves large amount of green color; residue of light greenish-tan color.
ethyl alcohol	dissolves large amount of green color; residue of light green color.
isopropyl alcohol	partial solution of green color; residue of light green color.
n-propyl alcohol	dissolves large amount of green color; residue of light gray green color.
n-butyl alcohol	dissolves some green color; residue of green color.
isoamyl alcohol	same as n-butyl alcohol.
diethyl ether	complete solution.
diisopropyl ether	complete solution.
acetone	dissolves small amount of green color.
methylisobutyl ketone	dissolves large amount of green color; residue in fine suspension.
ethyl acetate	dissolves some green color; residue in fine suspension.
methyl cellosolve acetate	dissolves some green color; residue still retains most of its green color.
butyl carbitol	gives fine, viscid suspension, dark green in color.

SOLVENT	EFFECT
dioxan	partial solution; residue in fine, viscid suspension.
pyridine	dissolves to great extent; with dark green color.
dec base	dissolves some green color; residue still retains most of its color.

From these results the methyl alcohol seemed to dissolve out most of the green color from the material, leaving the insoluble residue in a semi-solid state and very much lighter in color. Ethyl alcohol had the same effect. As a further experiment, a portion of the original material was dissolved in ether and to this was added methyl alcohol in excess. There resulted a precipitation of a semi-solid, light colored substance, with most of the green color remaining in solution. Upon repeating this experiment with ethyl alcohol the precipitation was not as complete and it resulted in a suspension of the precipitated substance which did not settle out readily.

The entire amount of dark green, sticky, semi-solid residue was washed in separate portions with a mixture of methyl and ethyl alcohols, allowing the insoluble residue to settle out, and decanting the supernatant green colored liquid. After about five liters of washings had been collected in this way, the residue remaining behind had become much lighter green in color. This was dried by allowing a stream of air to blow over it and evaporate the remaining alcohol mixed with it. Upon exposure to the air the residue darkened in color to a dark green. The alcohol washings were reserved for later study.

The saponification value of the residue was determined:

	I	II	Blank
Weight of sample	1.2062	2.2275	
Alkali added	25.00cc	25.00	25.00
Normality of alkali	0.5820		
HCl used to back titrate	23.30cc	20.24	24.78
Normality of HCl	0.5872		
Alkali to saponify	1.49cc	4.54cc	
Gms. KOH used	0.04899	0.14929	
Saponification Value	40.6	67.0	

The two saponification samples from the above were poured together and filtered. By this means the original residue was separated into three fractions:

(D1). The unsaponifiable, semi-solid, sticky residue, green in color, remaining behind in the flask. This was dissolved in ether and alcohol added just to the point of turbidity, and placed in an ice bath. After standing a short time there separated out a light yellowish-green colored substance.

(D2). The saponified material insoluble in the saponification liquid. This, when collected on the filter, was olive green in color and semi-solid. Upon dissolving it in hot alcohol and allowing to cool, the material separated out again in an apparently crystalline form.

(D3). The saponified material, soluble in the saponification liquid. This fraction was contained in the filtrate, which, upon evaporation of the alcohol and acidification with dilute HCl separated out in a light greenish-white form.

Saponification of Residue (D).

The entire residue from above was then saponified for 7 hours with an excess of alcoholic KOH. Upon cooling of the saponified product, the three fractions noted above were again separated:

(D1). The unsaponified residue, a semi-solid, sticky, greenish-brown material, which remained behind in the bottom of the container.

(D2). Saponified material insoluble in the saponification liquid. This was collected on a filter.

(D3). Saponified material soluble in the saponification liquid; this was contained in the filtrate.

(D1). The Unsaponified Fraction - Isolation of Hentriacontane, $C_{31}H_{64}$.

This fraction was treated with an excess of ether, in which most of it dissolved after long standing. Filtration separated the small amount of this fraction which was insoluble in ether. The ethereal filtrate was allowed to evaporate spontaneously, during which there separated a quantity of fine greenish-yellow colored crystalline material. Upon complete evaporation of the ether the residue was also greenish-yellow and crystalline in appearance, and sticky in consistency. It gave a color test for sterol[5].

Fifteen Gms. of this were refluxed for 6 hours with an excess of acetic anhydride. The mixture was then placed in a large test tube arranged with a wash bottle arrangement, and the whole placed in a bath of hot water. After standing in

this bath for a short time, there separated to the top a layer of dark greenish-brown colored residue, which was supposedly a hydrocarbon residue. The lower layer, supposedly containing sterol acetates in solution in the hot acetic anhydride, was siphoned off, and the hydrocarbon residue washed with fresh acetic anhydride, which was kept hot in the water bath. This washing process was continued until the washings gave no crystalline separation upon cooling. The hydrocarbon residue in the tube was then refluxed with fresh acetic anhydride for 3 hours more and the washing process repeated again, whereby an additional amount of sterol acetate was obtained.

The sterol acetates crystallised out of the acetic anhydride solution upon cooling. When collected upon a filter they were still contaminated with bits of hydrocarbon, so the above described washing with acetic anhydride was repeated on these impure acetates. The acetates, purified in this manner, and collected on a filter, were pale yellowish-green in color. Upon crystallisation from hot alcohol they were almost white in color, but when dried they assumed a distinct olive green color. The mother liquors were reserved for concentration. According to Rosenthaler, these mother liquors should contain the acetates of unsaponifiable alcohols (wax alcohols), these being more soluble in alcohol than the sterol acetates. Complete evaporation of the mother liquors, however, left no crystalline residue.

After observing the results with a small amount of the unsaponifiable residue, the remainder of this residue, 50 Gms.

in all, was refluxed with 55cc of acetic anhydride for 4 hours and then poured into a large test tube with the washing arrangement and immersed in a hot water bath as described above. Again there was a separation of the hydrocarbon layer to the top and the hot acetic anhydride solution to the bottom. However, the wash bottle arrangement being shown to be inadequate for such a large quantity of material, the material was washed further by heating with an excess of acetic anhydride on a hot plate for 1/2 hour and placing in an ice bath to cool, whereupon the hydrocarbon layer separated to the top, while the sterol acetate crystallised out in the bottom from the excess of acetic anhydride. The sterol acetate and excess acetic anhydride were then removed by making a hole in the hydrocarbon layer and pouring off the lower layer into a filter, where the solid sterol acetate was collected, while the excess acetic anhydride, in the filtrate, could be used for washing the hydrocarbon layer again. In this manner the unsaponified residue was separated into two fractions: The fraction soluble in hot acetic anhydride and the fraction insoluble in hot acetic anhydride.

The Fraction Soluble in Hot Acetic Anhydride:- This consisted of a cake of the sterol acetates admixed with some hydrocarbons, which was collected on a filter. This solid cake was heated with 3000 cc of alcohol, when it was observed that a portion of it did not dissolve but settled to the bottom in the form of an oily layer which solidified on allowing the alcohol to cool. Suspecting this insoluble portion to be

a hydrocarbon because of its insolubility, the 3000 cc of solution (I) were separated and the insoluble portion dissolved in an additional 3000 cc of hot alcohol (II). These two separate solutions were refluxed for 1/2 hour with charcoal in order to decolorise them, and then filtered through a steam heated funnel. Upon cooling to room temperature there appeared in both solutions flocculent white colored crystalline deposits. These were collected on filters. The deposit from solution (I) appeared flaky white, M.P. 62-8°, With the Liebermann-Burchard color test it gave after long standing a faint color reaction which was not the typical sterol color reaction. It was waxy to the touch, and upon standing with concentrated sulfuric acid gave a charring effect but little solution. The deposit from solution (II) was flaky white in appearance, retaining some of the yellow color from the mother liquor. It was waxy to the touch and of a very soft consistence; M. P. about 65°. The reaction to the Liebermann-Burchard test and to sulfuric acid was similar to that of the deposit from solution (I). The mother liquors in each case were reserved for the isolation of sterols.

The saponification values of these two materials were determined:

	Deposit (I)	Deposit (II)	Blank
Weight of sample	0.6000 Gms.	0.4771 Gms.	
KOH used to saponify	25.00 cc	25.00	25.00
Normality of KOH	0.4331		
HCl used to back titrate	17.42 cc	18.02	18.44

Normality of HCl	0.5872	
0.4331 N HCl used(equiv.amount)	23.62 cc	24.43
cc KOH used to saponify	1.38	.57
Saponification value	55.7	20.9

These values show that the materials contained a small amount of ester, hence they were almost free of sterol acetate. In order to free them from any sterol acetate the entire amounts of both of them were saponified for two hours with an excess of alcoholic potassium hydroxide. Upon cooling, the saponified product in each case formed a solid cake on the top. These cakes were filtered off and each dissolved in 600 cc of hot alcohol. Upon cooling the alcohol solution a pure white crystalline substance settled out in each case. When collected on a filter and allowed to dry these two products showed the following properties:

Product from	Deposit (I)	from deposit (II)
Melting point	66°	66-8°
Action with conc. H_2SO_4	slight charring; no solvent action.	same
with dimethylsulfate	slight coloration on long standing; no solvent action.	same
1 drop Br_2 in CCl_4	not decolorised.	same
Conc. HNO_3 and H_2SO_4	slight charring.	same

These tests indicated the products to be saturated aliphatic hydrocarbons. A small amount of each was treated with a mixture of concentrated sulfuric and nitric acids, poured into water and recovered, and then recrystallised

from hot alcohol with the aid of charcoal. They then showed the following properties:

	From Deposit (I)	From Deposit (II)
Melting point	66.5-67°	67.5°
Setting point	65.5-66°	67°
Melting point when taken simultaneously in same bath.	66.5-67°	67-67.5°

The corresponding properties given for the synthetic paraffin hydrocarbon, $C_{31}H_{64}$ by Piper and coworkers* are as follows:

Melting point	67.6-67.8°
Setting point	67.3°

These constants are in close agreement. In order to determine whether the product isolated in this case was one hydrocarbon or a mixture, samples of the two were sent to Dr. Sterling B. Hendricks, of the Bureau of Chemistry and Soils, Washington, D.C., for x-ray examination. The report on the crystal spacings was as follows:

	From Deposit (I)	From Deposit (II)
A spacing d (001)	41.00±0.20A°	41.45±0.25A°

The corresponding spacing observed by Piper for the synthetic hydrocarbon, $C_{31}H_{64}$, is 41.55A°. This close agreement of constants proves that the hydrocarbon isolated above is not a mixture but is a fairly pure specimen of hentriacontane, $C_{31}H_{64}$.

* Biochemical Journal 25,2080(1931).

The Mother-Liquors after Crystallisation of the Hydrocarbon - Isolation of Sterols.

These mother-liquors were combined and allowed to concentrate by evaporation to a volume of 200 cc, during which time there was a separation of a yellow colored, oily appearing material. When it was attempted to collect this material on a filter it was absorbed by the filter and could not be handled. This material gave the color test for sterols. Because of the small amount it was collected by dissolving it off the paper in ether and the ethereal solution reserved. The mother-liquor upon complete evaporation yielded more of the material which was added to the ether solution. The ether solution was then allowed to evaporate spontaneously and left behind a pale yellow semi-solid residue. This was dissolved in the smallest amount of hot alcohol, hot water added to the point of incipient turbidity, and the solution cooled in an ice bath. A pale yellow solid material separated, which, upon collection on a filter became semi-solid again. Repeated attempts to crystallise this material from alcohol and from hydro-alcohol solutions were unsuccessful. Attempts to purify the material by precipitation from alcoholic solution with digitonin were also unsuccessful.

The Fraction of the Unsaponified Residue Insoluble in Hot Acetic Anhydride - Isolation of a Caoutchouc-like Substance.

This consisted of the residue left behind after the removal of the sterols and hydrocarbons and was represented by a brown colored, semi-solid, very sticky layer which remained

insoluble in the hot acetic anhydride during the treatment previously described for the separation of the sterols and hydrocarbons. This material was refluxed with an excess of alcohol for an hour in order to remove any hydrocarbon or sterol that might have remained behind. It was observed that the material was quite insoluble in alcohol. The alcoholic filtrate upon evaporation left an insignificant residue, showing very little hydrocarbon or sterol had remained. This material was soluble in ether and benzene. It was dissolved in a slight excess of benzene and to this solution alcohol was added to purify it. The material thus obtained was of a greenish-brown color and very tenacious, resembling the stretching properties of rubber. With the hope of removing the coloring matter by washing a petroleum ether solution of it with furfural, its solubility in petroleum ether was tested and it was found to be insoluble in this solvent.

Action of concentrated sulfuric acid- A small amount of this material was treated for several days with concentrated sulfuric acid, the mixture them poured into water, and the insoluble residue collected on a filter. Obtained in this way the residue was dark brownish-black in color, and brittle in appearance.

(D2). The Saponified Material Insoluble in the Saponification Liquid - Isolation of a Mixture of Paraffin Hydrocarbons.

This, when collected on the filter, was grayish-green and crystalline in appearance. It was dissolved in a large

amount of hot alcohol, filtered, and the filtrate allowed to cool, whereupon the material crystallised out again. The crystalline material was collected by suction filter and when dried gave no color test for sterols. It was insoluble in water, dilute acid and alkali, almost entirely insoluble in concentrated sulfuric acid, giving only a slight amount of charring. It burned with a luminous flame, leaving a small amount of black residue. It softened around 250-75° and melted completely at about 295°. Its color was an olive drab.

One gram of this material was treated with 50 cc of concentrated sulfuric acid for 4 hours and the mixture then poured into water. This resulted in the precipitation of a light grayish-white colored material, which when collected on a suction filter and dried, assumed a light brown color, and finally the olive drab color of the original material before such treatment. The weight of material re-obtained was more than 0.9 Gms., and melted partially at 80-6° and completely around 200°. The filtrate from the acid treatment was boiled with barium carbonate and filtered. The filtrate upon cooling showed nothing of a crystalline nature separating and was discarded.

In another experiment 1/2 gram of the material was dissolved in 100 cc of hot alcohol and the solution refluxed with bone charcoal for 15 minutes and filtered. The filtrate upon cooling deposited a white gelatinous material which when dried was grayish-white in color. It melted partially at 95-100° and completely at 175-80°, indicating it to be a mixture.

This last experiment having indicated a method of purifying the original material, the remainder of this fraction was dissolved in 800 cc of hot alcohol, refluxed with charcoal for 1/2 hour, and filtered through a steam heated funnel. The hot filtrate upon cooling deposited a white appearing substance. When collected on a filter and dried this substance was grayish-white in appearance.

In an attempt to find if this assumed mixture contained anything of an alcoholic nature a small amount of it was refluxed for 2 hours with acetic anhydride. During this treatment it was observed that two layers were formed, the lower layer consisting of acetic anhydride with some dissolved material, the upper layer being insoluble in the acetic anhydride and remaining in an oily form on top. Upon cooling, the lower layer assumed a light tan, semi-solid appearance. With a thought of using this means as a method of separation of the mixture, the entire amount was then refluxed for 3 hours with acetic anhydride, the two layers being observed again as above. The hot mixture was then poured into a convenient sized test tube and allowed to cool, whereupon the two layers were formed again. The upper white, brittle layer was separated from the lower tan, semi-solid layer mechanically.

The upper layer- This was dissolved in 700 cc of hot alcohol, filtered through a hot funnel, and the filtrate cooled in the refrigerator, whereupon a white colored gelatinous mass separated out. When collected and dried it was pure white in appearance and melted partially at 70-1°, completely at 107°,

indicating it to still be a mixture. This material was treated for two days with an excess of concentrated sulfuric acid in an effort to remove any impurities. The acid mixture assumed a tan color, showing slight impurities to be present. It was poured into an excess of water, the solid material filtered off and dried. It then melted almost entirely at 70-5° and completely at 85°. This material, which now had a light tan color, was dissolved in 900 cc of hot alcohol, filtered through a hot funnel, and the filtrate allowed to cool, when the material separated out again in a pure white form. This was collected and dried.

The lower layer- This tan colored, semi-solid layer was placed in 100 cc of water and agitated to decompose any excess acetic anhydride. The mixture was then filtered. The tan colored residue on the filter when dry softened at 71° and melted completely at 81°. This material was also treated with concentrated sulfuric acid to remove impurities. The acid mixture assumed a light brown color, indicating some impurities. It was poured into an excess of water, the insoluble residue filtered off and recrystallised from hot alcohol with the aid of charcoal. The product thus obtained had the same melting point as before this treatment.

The upper and lower layers having been shown to have approximately the same melting points, they were placed together and the sulfuric acid treatment described above repeated on this mixture. Thus obtained, the product melted partially at 69.5-70° and completely at 74°, indicating it to be a mix-

ture of paraffin hydrocarbons. The amount at hand was too small to allow any further attempts to separate the mixture.

(D3). The Saponification Material Soluble in the Saponification Liquid.

This fraction consisted of the solution of the potassium soaps of fatty acids, An equal amount of water was added and the alcohol reoved by evaporation on the water bath. Upon acidification of the aqueous solution with concentrated hydrochloric acid, the free fatty acids precipitated in a dark green, sticky residue. These were separated from the aqueous portion mechanically, and the aqueous portion reserved for tests for glycerol.

The mass of free fatty acids was neutralised to phenolphthalein with potassium hydroxide and to this was added, with brisk stirring, 75 grams of lead acetate dissolved in 300 cc of boiling water. There resulted a dark green precipitate of the lead salts, which clung to the sides of the beaker. The stirring was continued until the beaker full of contents was cold. After drying in vacuo, the lead salts were refluxed with an excess of ether for 1 hour, allowed to stand in the refrigerator overnight, and filtered. The residue on the filter was again refluxed with ether, and this procedure repeated until the ethereal filtrate no longer contained a green color. The lead salts of the fatty acids were thus separated into two fractions; the lead salts of the liquid fatty acids, soluble in ether, and the lead salts of the solid fatty acids, insoluble in ether.

Solid Fatty Acids - Isolation of Myristic Acid.

The lead salts insoluble in ether were treated under ether with excess 25% hydrochloric acid to decompose the lead. The ethereal solution of the fatty acids was filtered from the resulting lead chloride precipitate and washed with water until the washings were no longer acid to litmus. The ethereal solution was then allowed to evaporate, leaving a solid cake of fatty acids weighing about 4.5 grams. The neutralisation value of this mass was determined:

Weight of sample	0.1088	0.1932
NaOH used to neutralise	3.54 cc	6.32 cc
Normality of NaOH	0.1076	
Neutralisation Equivalent	285.6	284.1

The cake of fatty acids was dissolved in 200 cc of hot methyl alcohol, refluxed with charcoal for 15 minutes, filtered, and cooled in an ice bath. There crystallised out in granular form a small quantity of light cream colored acid. The filtrate was allowed to concentrate spontaneously to one-half its volume.

First Fraction of solid acids- The cream colored material softened at 51° and melted at 54°. It was recrystallised from hot methyl alcohol, when it came out in pure white appearance, with a melting point of 53-4°. The neutralisation equivalent of this acid was determined:

Weight of sample	0.097
NaOH to neutralise	3.16 cc
Normality of NaOH	0.1076

Neutrality Equivalent 285.3

The p-bromphenacyl ester of this acid, when first prepared, melted at 72-3°. Upon recrystallisation it melted at 79-80°. Myristic acid melts at 54° and its p-bromphenacyl ester melts at 81°. From these results the acid in the first fraction was identified as myristic acid. The neutralisation equivalent does not confirm this conclusion. (Neutralisation Equivalent for myristic acid is 228). However, this difference in the neutralisation equivalent may be partially explained by the possibility that the acid was not entirely dry and that the original acids were not washed completely free from mineral acid.

Second Fraction of solid acids- This fraction, collected from the mother liquor of the first fraction after concentration to one-half its volume, was light cream colored and melted at 52-7°. When recrystallised from a solution of methyl alcohol and water (9:1) it was obtained as a pure white substance, melting at 55-7°. The amount was too small to admit of any further characterisation.

Liquid Fatty Acids - Identification of Oleic Acid.

The lead salts of the liquid fatty acids were contained in the ethereal filtrate. An excess of 25% hydrochloric acid was added to this solution to decompose them and the ether solution of the free fatty acids filtered from the lead chloride precipitate and washed with water until the washings were no longer acid to litmus. The ethereal solution was then dried over anhydrous sodium sulfate and allowed to evaporate spon-

taneously for the removal of the ether. Of 550 cc of the ethereal solution, 55 cc were evaporated to constant weight and dried, to obtain a measure of the amount of liquid acids:

Weight of liquid acids in 55 cc	2.3099
Weight of total liquid acids obtained	23.089 Gms.

The ether solution of the liquid fatty acids was then treated to separate the liquid acids by the lead salt-ether method. To the ether solution 300 cc of glacial acetic acid were added and the mixture cooled to -10°. Bromine was then allowed to drop into the solution with constant stirring until it had aquired an excess as noted by the color and odor. It was then allowed to stand in the refrigerator overnight. At this time a small amount of a light yellow precipitate was deposited. When collected and dried, this appeared to be largely inorganic in nature, leaving behind a large amount of ash on burning. It did not show the solubility nor melting point characteristic of the linolenic acid hexabromide which should be obtained at this point if linolenic acid were present. It was insoluble in ether, benzene, alcohol, and darkened, but did not melt below 200°. It gave a slight test for halogen and no test for sulfur.

After removal of this precipitate the ether solution was washed with a saturated solution of sodium thiosulfate to remove the excess of free bromine. The excess sodium thiosulfate was in turn removed by washing with water, and the ether solution then dried over anhydrous sodium sulfate. The ether was evaporated and the residue treated with hot petroleum

ether. After standing overnight in the refrigerator, a light yellow colored precipitate had formed, which, when collected on the filter, turned dark brown in color. Attempts to recrystallise this from benzene, or an ether-petroleum ether mixture were unsuccessful. It was then treated with boiling alcohol, which dissolved the greater part of it; hot water was added to the filtrate until it became faintly turbid, and the solution was then allowed to cool in an ice bath. A light yellow precipitate settled out. This precipitate did not melt below 200°, and could not be identified as the linoleic acid tetrabromide that should be obtained at this point.

The petroleum ether solution, after the removal of the precipitate as noted above, was evaporated spontaneously, the residue taken up in 100 cc of alcohol and heated under reflux with 5 grams of zinc filings for four hours to regain the oleic acid from its dibromide. At the end of this time the alcohol was removed by evaporation, the residue poured into an excess of water, 10 cc of dilute sulfuric acid added, and the mixture heated on the water bath for 30 minutes. At the end of this time the oleic acid and any ester that might have been formed was extracted with ether, the ether evaporated, and the residue heated with alcoholic potassium hydroxide to saponify any ester. The saponified mixture was then poured into water, the alcohol removed by evaporation, the potassium salt acidified, the free acid shaken out with ether, dried over anhydrous sodium sulfate, and the ether evaporated, leaving a dark brown oily residue. This oily residue gave the elaidin test and the

vanillin color test for oleic acid. It was then treated with an aqueous solution of potassium hydroxide to form the soluble salt. This treatment did not result in a clear solution but rather in a grayish-green milky appearing mixture. To this was added, slowly and with mechanical stirring, a 2% aqueous solution of potassium permanganate with the aim of preparing the dihydroxy stearic acid for further identification. During this addition the mixture became first green in color, and later, when an excess of the permanganate had been added, it became dark brown in color, with the separation of a large amount of dark brown colored precipitate. This mixture was then acidified with sulfuric acid, for the purpose of dissolving the manganese dioxide formed and allowing the insoluble dihydroxy-stearic acid to remain behind. The dark brown precipitate which had formed did not dissolve, however. This was collected on a filter, and when allowed to stand became almost black in appearance and resin-like and sticky. Attempts to crystallise this material with various solvents were unsuccessful as was also an attempt to dissolve it in alkali. The black mass was then dissolved in 200 cc of petroleum ether and this solution shaken out three times with 20 cc of furfural each time. This treatment resulted in the colored substance settling out of the petroleum ether layer into the furfural layer. The petroleum ether solution thus obtained was perfectly clear and faintly green in color. Upon evaporation of the solvent there was left a small amount of a pale green oily residue.

Further attempts to characterise this residue were un-

successful. It did not dissolve in alkali, and another attempt to oxidise it with potassium permanganate solution resulted in the formation of the same black mass which was noted above. From the elaidin test and the vanillin color test the presence of oleic acid was indicated in this portion of the liquid fatty acids, but oleic acid itself, or any of its derivatives or oxidation products, could not be isolated.

Identification of Glycerol.

The mother liquor from the fatty acids, after they had been precipitated from an alkaline solution by the addition of hydrochloric acid, was allowed to evaporate to dryness on the steam bath. The residue remaining was extracted with a mixture of alcohol and ether, and the solvents were evaporated. A small amount of a thick syrup remained which had a sweet taste and was soluble in water. When heated with a small amount of potassium bisulfate this syrup gave the characteristic pungent odor of acrolein, showing it to be glycerol.

Extraction With Acetone.

Fifteen hundred grams of Uva Ursi leaves, after having been extracted with ether and petroleum ether, respectively, were next extracted with acetone in a large extractor of the Soxhlet type. The acetone extract, which was dark brown in color, was collected in six separate portions. In this way a total of approximately 10 liters of acetone extract were obtained.

Crystallisation of Arbutin from the Extract.-

In the first portion of the acetone extract there was

deposited, upon standing for a few days, a quantity of crystalline material on the sides of the flask. This was collected and freed from adhering color by washing with acetone. The mother liquor was placed in a refrigerator for a few days, during which time an additional amount of crystals was deposited. These were collected and the mother liquor then placed outside in a freezing atmosphere for several days. A large amount of crystalline material separated from the extract during this time. The mother liquor was then concentrated to a small volume by distillation of the acetone and this concentrated liquor, upon standing in the cold for several weeks, deposited an additional amount of crystals.

All of the different crops of crystals collected above, when washed with acetone and allowed to dry, were almost pure white in appearance. They melted at 193.5-4.5°, gave a positive test for glucosides, and gave the Jungmann color test for arbutin. A quantity of them was hydrolysed with dilute hydrochloric acid and the hydrolysate extracted with ether. Evaporation of the ether gave crystals of hydroquinone, M. P. 69°, identified by its acetate and benzoate. This showed the crystalline material to be arbutin, a conclusion which was confirmed by preparing the acetate and benzoate of the crystals themselves, which agreed in melting point with the corresponding derivatives of arbutin. The total amount of crystalline arbutin obtained in this way from the first portion of the acetone extract was approximately 20 grams.

Freezing of the Remainder of the Extract - Collection of Deposits Containing Tannin.

The remaining portions of the acetone extract collected above were allowed to stand in a freezing atmosphere for several weeks. During this time there was deposited in them large quantities of reddish-brown, solid deposits. This deposited material was collected and when dried it weighed approximately 75 grams. When reduced to a powder it was light tan in color. A tannin determination on this material, by the potassium permanganate method of Loewenthal*, gave the following results:

A 0.5000 gram sample was made up to 100 cc with water and 10 cc of this solution was used for titrations.

$KMnO_4$ used for 10 cc plus 20 cc of Indigo Carmine before 17.38 cc

$KMnO_4$ used for 20 cc of Indigo Carmine alone 9.32

$KMnO_4$ used for 10 cc of solution before hide powder. 8.06 cc

$KMnO_4$ used for 10 cc plus 20 cc of Indigo Carmine after. 11.20 cc

$KMnO_4$ used for 20 cc of Indigo Carmine alone 9.32

$KMnO_4$ used for 10 cc of solution after hide powder. 1.88 cc

$KMnO_4$ used for 10 cc of tannin solution 6.18 cc

Titer of $KMnO_4$ - 1cc equiv. to 0.001567 grams of tannin.

Total grams tannin in 100 cc - 6.18 x 0.001567 x 10 = 0.09684 grams.

Per Cent tannin - 19.37%, calculated as U.S.P. tannic acid.

* Villavecchia, V., Applied Analytical Chemistry, V.2, p.338, 1918.

Attempts to Separate the Tannin and Non-Tannin Constituents.

The following experiments were carried out on separate portions of the tannin-containing material collected above in an attempt to find some method for isolating the tannin from the non-tannin components of the mixture.

(a). Precipitation From Alcoholic Solution With Ether.

Ten grams of the material were dissolved in 100 cc of warm alcohol, allowed to cool, and to this cooled solution ether was added slowly with stirring. There occurred a flocculent precipitation, at first almost white in appearance, later turning to a light brown color. About 400 cc of ether were required for complete precipitation. The precipitate, upon allowing it to stand, settled out in a compact sticky mass. The supernatant liquor was drained off and allowed to concentrate slowly. The precipitate was dried in vacuo. A tannin determination on this precipitate showed it to contain 17.56% tannin. Thus as a means of accomplishing the desired purpose this method did not offer great possibilities.

The mother liquor from the precipitate, upon complete evaporation, left a considerable amount of crystalline material along with a sticky brown amorphous substance. This upon examination proved to be crystals of arbutin contaminated with a small amount of tannin.

(b). Extraction With Ethyl Acetate.

Ten grams of the material were extracted in a small Soxhlet extractor with ethyl acetate for 72 hours. At the

end of this time the residue in the extractor had become a gum-like mass. The liquid extract obtained was allowed to concentrate slowly and when completely dry it left a small amount of colorless crystalline substance which proved to be arbutin.

(c). Precipitation With Sodium Chloride.

Ten grams of the material were dissolved in 75 cc of water and to this solution were added 100 cc of a saturated solution of sodium chloride, slowly and with stirring. A light colored, flocculent precipitate was formed, which upon settling became light brown in color. The supernatant liquid, which was light brown in color, was poured off, the precipitate suspended in 50 cc of water, and 50 cc of sodium chloride solution again added with stirring. The precipitate was this time collected on a suction filter, washed with the sodium chloride solution, dried, and then taken up in absolute alcohol, the adhering sodium chloride remaining undissolved in this solvent. Upon evaporation of the alcohol and drying the residue it was found that it was insoluble in water. Since all of the well known tannins are water soluble this property did not characterise the residue as a tannin. It was found to be soluble to some extent in hot water, but on cooling a reddish tan colored, flocculent material settled out again. This behavior is characteristic of phlobaphenes and indicated that this residue might be of that nature.

(d). Precipitation With Neutral Lead Acetate.

Twenty grams of the material were dissolved in 100 cc of water and to this solution was added a saturated solution of

neutral lead acetate, slowly and with stirring, until no more precipitation occurred. The resulting precipitate was light yellow in color. It was collected and washed with water.

The Filtrate.- The filtrate, together with the washings, was almost colorless. The addition of basic lead acetate solution to a test portion gave no further precipitation. The excess lead was removed from this filtrate with hydrogen sulfide and the excess hydrogen sulfide removed by gentle boiling. The filtrate gave a positive test for reducing sugars. It was allowed to concentrate slowly at room temperature to a clear, light brown colored syrup of the consistency of molasses. Nothing of a crystalline nature separated after long standing. It gave the Jungmann test for arbutin and also a heavy reduction with Fehling's solution. With phenylhydrazine it formed an osazone after standing in boiling water for 3-4 minutes. After recrystallisation the osazone melted at 205-7°, indicating glucose to be present. Repeated attempts to crystallise a sugar from this residue by allowing it to cool from various solvents were unsuccessful.

The Lead Precipitate.- This precipitate was suspended in water and 10% sulfuric acid was added to decompose the lead. After the addition of excess acid there still remianed a quantity of light red colored precipitate which was not all lead sulfate, indicating that some of the regenerated products from the lead precipitate were not water soluble. The soluble portion was removed by filtration, the filtrate being a reddish-brown color. The residue remaining was then extracted with hot

water and finally with hot alcohol, both of these solvents removing most of the residue. The hot water extract upon cooling deposited a considerable quantity of a tan colored, amorphous material which assumed a red color on standing. The cold water filtrate from above also deposited a red colored substance on standing. The substance obtained in this way had the appearance and solubility properties of a phlobaphene. Its formation might be explained by the action of the excess sulfuric acid on the tannin regenerated from the lead precipitate.

Concentration and Freezing of the Mother Liquors From the Acetone Extract. - Collection of More Tannin-Containing Deposits.

After the removal of arbutin and the tannin-containing deposits from the acetone extract as described above, the mother liquors from all of the portions of the extract were concentrated by distilling off the acetone, and the concentrates allowed to stand in a freezing atmosphere for several weeks. This resulted in the deposition in these liquors of an additional amount of material which was partially crystalline in appearance and partially semi-solid and sticky. This material, when collected from all of the concentrates and dried, was light brown in color and weighed about 62 grams. A tannin determination on this material showed it to contain approximately 19% tannin.

Precipitation of the Concentrated Acetone Mother Liquor With Ether.

The concentrated mother liquor obtained from above was

dark brown in color and of the consistency of a thin syrup. It amounted to about 500 cc. To this liquor was added 300 cc of ether with the result that a dark brown material, of a very thick consistency, was precipitated and settled out. When collected and dried this material was light brown in color and weighed 108 grams. A tannin determination on this showed it to contain 23% tannin.

The addition of more ether resulted in the precipitation of another fraction of the above material. Complete precipitation had occurred after 1200 cc of ether had been added. The second fraction thus precipitated weighed 43 grams. A tannin determination showed it to contain 28.3% tannin.

Determination of Reducing Sugars Before and After Hydrolysis.

Five grams of the first fraction of material precipitated from the concentrated acetone mother liquor by the addition of ether were used to determine reducing sugars before and after hydrolysis with dilute sulfuric acid. The Defrens O'Sullivan* method was used:

Weight of sample 5.0000 grams. Sample was dissolved in water, cleared of tannins by the addition of a saturated solution of lead acetate, the excess lead removed with potassium oxalate solution, and the filtrate made up to 100 cc. Ten cc of this solution were then made up to 100 cc and 25 cc of this solution were then used for the determinations:

* Schuette,H.A. & Oppen,F.C., Principles of Organic Analysis, p.10,1935.

Weight of crucible plus CuO	10.747	10.409
Weight of crucible alone	10.715	10.376
Weight of CuO	0.032	0.033
Average	0.033	

From Defren O'Sullivan Tables, 0.033 grams CuO is equiv. to 14.5 mg. of glucose.

Total grams of glucose in original 100 cc of solution = 0.0145 x 4 x 10 = 0.580 grams.

Per cent reducing sugars, as glucose- 11.60%

Twenty five cc of the original 100 cc of solution were then refluxed with 10 cc of 10% sulfuric acid for one hour to hydrolyse any glucoside. The hydrolysate was made up to 100 cc and 10 cc of this solution were then used for the determination:

Weight of crucible plus CuO	10.855	10.517
Weight of crucible alone	10.715	10.376
Weight of CuO	0.140	0.141
Average	0.141	

From Tables, 0.141 grams CuO equiv. to 62166 mg. of glucose.

Total grams of glucose in original 100 cc = 0.06266 x 10 x 4 = 2.5064 grams.

Per cent reducing sugars, as glucose- 50.13%

From these results it may be seen that free reducing sugars, as well as a large amount of glucosides, presumably arbutin, was contained in the material precipitated from the acetone extract by the addition of ether. It should be pointed out, however, that hydroquinone, yielded upon the hydrolysis of arbutin, also reduces alkaline copper tartrate solutions and that this would tend to make the results obtained above abnormally high after hydrolysis of the arbutin.

Isolation of the Tannin With Lead Acetate.

The 43 grams of material obtained in the second frac-

tion of the precipitate above were dissolved in 1000 cc of water and to this solution was added a 10% solution of neutral lead acetate, slowly and with stirring, until a precipitate no longer formed, The resulting lead precipitate was bright yellow in color. It was centrifuged to facilitate filtering and then collected on a filter and washed thoroughly with water.

The Lead Precipitate.- This precipitate when allowed to dry weighed 32 grams. It was reduced to a powder, suspended in a pasty mass in 300 cc of alcohol, and hydrogen sulfide was passed through this suspension for several hours. The lead sulfide was then filtered off, excess hydrogen sulfide removed from the filtrate by bubbling carbon dioxide through it, and the filtrate then allowed to evaporate and dried finally in vacuo. The product thus obtained, consisting of the impure tannin, weighed 11.5 grams.

Properties of the Tannin.

This product was light brown in color, was soluble in water, alcohol, and acetone, and insoluble in ether and ethyl acetate. It gave a blue-green color with ferric ammonium sulfate test solution, gave no precipitate with bromine water, and did not give the Griessmayer color test for ellagic acid or ellagitannins.

Pyrolysis of the Tannin.

One gram of the tannin was heated to 160° in 5cc of glycerin, and then the temperature was slowly raised to 200-10° and kept there for 30 minutes. At the end of this time it was allowed to cool, diluted with a small amount of water, and

shaken out repeatedly with ether. The ether upon evaporation left a small amount of a light yellow, non-crystalline material. In aqueous solution this substance gave no precipitate with bromine water, gave a blue-black color with ferric ammonium sulfate solution, and a violet color with lime water, indicating the presence of pyrogallol. Upon evaporation the aqueous solution left a white crystalline substance, melting at 130-3°. The amount obtained was too small to identify conclusively as pyrogallol by the preparation of derivatives.

Dry Distillation of the Tannin.

One gram of the tannin was placed in a large test tube with a side arm attachment and heated in an oil bath, at the same time evacuating the test tube to a high vacuum with a suction pump. At about 190-200° a small amount of white crystalline substance began to deposit on the upper walls of the tube. The heating was continued until the residue in the bottom of the tube was completely charred. The crystals were collected and had a melting point of 130-3°. They also gave a blue-black color with ferric ammonium sulfate solution and a violet color with lime water, showing the crystalline decomposition product of the tannin to be pyrogallol.

Preparation of Uva Ursi-Tannin by Nierenstein's Method.

Nierenstein* reported, in an investigation of the tannin of the Knopper Gall (Quercus cerris) that he was able to extract the tannin with acetone, from which solution it was precipitated upon the addition of petroleum ether (light petroleum). This

*Nierenstein,M., J. Chem. Soc. 115,1174,1919.

method was carried out on Uva Ursi in the following way:

One thousand grams of Uva Ursi were extracted in a Soxhlet type continuous extractor with chloroform to remove fats and green coloring matter. After this extraction, the drug was thoroughly dried and was then extracted with acetone for four days. The acetone extract was filtered, separating the liquid portion from a brown, semi-solid portion which had settled out of the liquid extract. It was then made up to 1500 cc with acetone and to this was added 400 cc of petroleum ether (B.P. 60-80°). There resulted the separation of a light tan colored precipitate which upon standing darkened slightly in color and became partially crystalline in appearance. This precipitate was separated by decantation. A tannin determination on the dry material showed it to contain 36.3% tannin.

Suspecting the crystalline part of the precipitate to be arbutin, it was desirable to find some means of separating this from the tannin. Fifty grams of the precipitate were dissolved in 250 cc of alcohol and to this solution was added 50 cc of petroleum ether. No precipitation resulted and the further addition of petroleum ether up to 200 cc still gave no precipitation. The tannin was evidently quite soluble in the mixture of alcohol and petroleum ether. This solution was then allowed to evaporate to dryness to get back the original precipitates.

Attempt to Remove the Tannin With Hide Powder.

Ten grams of the precipitated mass obtained above were dissolved in 75 cc of water, 5 grams of hide powder added to

the solution, and this mixture shaken frequently during 24 hours. At the end of this time it was filtered by suction and the hide powder, collected in the funnel, washed with water until the washings no longer gave a color with ferric chloride solution. The hide powder upon drying did not show any of the properties of a tanned hide. The filtrate, which was light brown in color, gave a precipitate with 1% gelatin solution, indicating that the hide powder did not remove all of the tannin. Treatment of this filtrate with charcoal failed to remove any of the color. It reduced Fehling's solution upon long boiling. When heated with dilute acid for a short time it reduced hot Fehling's solution almost immediately, indicating the presence of glucosides. It also gave a positive test for arbutin. When allowed to evaporate to dryness it left a dark brown residue in which crystals of arbutin were imbedded.

Removal of the Tannin With Magnesium Oxide.

Ten grams of the mass precipitated from the acetone extract were mixed intimately with an equal weight of magnesium oxide and the dry mixture moistened with water. A bright yellow color developed at once. This thoroughly mixed and moistened mass was allowed to dry on a steam bath for one hour and then extracted with hot alcohol.

The Alcoholic Extract.- This was a light tan colored liquid and no longer gave a precipitate with gelatin solution, indicating that the tannin had been removed from it. Upon evaporation of the alcohol there was left a considerable quantity of almost colorless crystalline material which melted

partially at 120-40° and completely at 189-92°. When dried for one hour at 110° it melted partially at 144-6° and completely at 189-92°. Arbutin has been reported in the literature to have two melting points, 142-3° and 194-8°. Van Rijn* has stated that air dried arbutin melts at 142-3° while the anhydrous form melts at 194-5°. Refluxing the above material with acetic anhydride for two hours gave a white silky crystalline compound melting partially at 144-6° and completely at 190-200°. Refluxing this compound with acetic anhydride for an additional three hours yielded a compound melting completely at 144-5°, corresponding to the melting point of pentaacetyl arbutin. From these results it was concluded that arbutin was the only substance contained in the acetone extract after the tannin had been removed with magnesium oxide.

The Magnesium Oxide Residue.- This residue was light brownish-yellow in color. A part of it was suspended in water and the magnesium oxide decomposed by the addition of hydrochloric acid. When all of the oxide had been decomposed and the solution had become acid in reaction there was precipitated a brick red material from the solution. When collected on a filter and allowed to dry this substance resembled a phlobaphene in its color and solubility. It was insoluble in cold water, but soluble in alcohol and in hot water, from which solvent it precipitated again on cooling. In aqueous or alcoholic solution it gave a blue-green color with ferric chloride solution. Attempts to crystallise this substance from

*VanRijn, Die Glycoside, Ed.2,p.373,1931.

various solvents were not successful.

A Detannated Fluidextract of Uva Ursi.

Wruble* prepared a completely detannated tincture of cinchona by mixing the powdered drug with an equal weight of calcium hydroxide and then percolating this mixture with 95% alcohol. His experiment was repeated with Uva Ursi in the following way:

Five hundred grams of Uva Ursi were intimately mixed with 500 grams of freshly slaked lime, the mixture moistened with 95% alcohol, allowed to stand in a closed container for 6 hours, packed in a percolator, and allowed to macerate with alcohol for 48 hours. Five hundred cc of a fluidextract were then made, according to U.S.P. directions, using alcohol as a menstruum.

At the same time 250 grams of untreated Uva Ursi were used to make a fluidextract with alcohol as a menstruum, this fluidextract to be used as a control sample.

Tannin determination of the two fluidextracts.- This determination was carried out by the indirect, hide powder method, according to directions given in Villavecchia**.

	Control Sample	Lime-treated Sample
Weight of sample	15.7927	17.4137
Sample made up to	500 cc	500 cc
Total solids before hide powder.	0.4850(in 50cc)	0.2600(in 100cc)

*Wruble,M., J.Am.Pharm.Assoc.23,208,1934.

**Villavecchia,V., Applied Analytical Chemistry 2,337, 1918.

the two formulas in question. It may be pointed out that the compound analysed by Van der Haar had a melting point of 279-80°, while that of Sando melted at 284-5°, showing that the question of purity may have influenced the results obtained. On the other hand, however, the criticism of macro-combustion of such high molecular compounds which was made by Van der Haar seems to be a just one which is held by other investigators.[32] However, the question of the molecular formula of ursolic acid, on the basis of evidence presented thus far, should still be regarded as an open one. Winterstein and Stein,[27] in 1931, carried out both combustion analyses and titration with standard alkali upon ursolic acid and its derivatives. They observed that the results of their combustions agreed with the $C_{30}H_{48}O_3$ formula, while titration values agreed almost equally as well for the $C_{31}H_{50}O_3$ formula. These authors pointed out that a choice between the two formulas is important from the phytochemical standpoint in gaining an insight into the formation of ursolic acid and its relation to other closely related plant constituents. By assuming for it the formula, $C_{30}H_{48}O_3$, ursolic acid could be conceived as an oxidation product of a triterpene alcohol, $C_{30}H_{49}OH$, such as the amyrins, while with the formula, $C_{31}H_{50}O_3$, the assumption might be made that the triterpene alcohols are formed through decarboxylation of the corresponding carboxy acids.

In the following table is given a summary of the results obtained by various investigators from the combustion analysis of ursolic acid. Results of the analysis of the numerous derivatives of the compound would require too much space to be given here and are no more enlightening in the problem at hand. These results may be found by referring to the original literature which is cited.

Sectional Percolation Studies on Uva Ursi

Introduction

In order to study the process of percolation and to follow the changes occurring in the menstruum as it passes from one stratum of drug to the other in its course down through the percolator, Wruble,[1] in 1933, developed the technique of sectional percolation. The best way to study the menstruum at various points along its downward path through the percolator would have been to segment the drug in the percolator into a number of equal layers and draw off samples of the percolate at the bottom of each segment. This, however, could not be done in a practicable manner. In order to approach such an experimental method Wruble made use of a number of percolators, each representing a hypothetical segment of a large percolator. The amount of drug in each succeeding percolator differed from the preceeding one by a regular decrement. A definite amount of percolate was collected from the first percolator, a portion of this reserved for tests, and the remainder used to percolate a second portion of the drug, representing the second hypothetical segment of a large percolator. Thus, using cinchona bark, Wruble started with 1000 grams in the first percolator, 900 grams in the second, 800 grams in the third, and so on to the tenth percolator which contained 100 grams. From the percolator containing 1000 grams, 1000 cc of percolate were collected, 100 cc of this amount reserved, and the remaining 900 cc were used to percolate the second segment of 900 grams of fresh drug. In this way a total of ten 100 cc samples of percolate were reserved, each of which represented, theoretically, a sample of

the percolate as it would be obtained after passing through each successive layer of drug from the top to the bottom of a large percolator.

Using the same technique, Powers,[2] in 1936, repeated Wruble's sectional percolation studies on cinchona bark of a different source and alkaloidal content.

Experimental

Inasmuch as both Wruble and Powers observed certain anomalies in their studies on the sectional percolation of cinchona, it seemed desirable to repeat their experiments, using Uva Ursi as the drug. The procedure used was essentially the same as that of Wruble described above. Two thousand seven hundred and fifty grams of Uva Ursi were divided into ten parts. The first portion weighed 500 grams, the second 450 grams , the third 400 grams, and so on down to the tenth, which weighed 50 grams. The 500 gram portion was moistened with 375 cc of 95% alcohol and allowed to stand in a closed container for 6 hours. It was then packed in a percolator, enough alcohol added to saturate the drug, the percolator covered, and the drug allowed to macerate during 48 hours. At the end of this time percolation was started and the rate of flow from the percolator carefully regulated. The menstruum used to percolate this 500 gram portion was 95% alcohol.

Five hundred cc of percolate were collected from this 500 gram portion. Of this amount 50 cc were reserved. The second portion of drug, 450 grams was then moistened with a proportionate amount of alcohol, packed in a percolator under

the same conditions as was the first portion, allowed to macerate, and then percolation was started, using as a menstruum the 450 cc of percolate collected from the first portion. From this second portion 450 cc of percolate were collected, 50 cc reserved, and the remaining 400 cc used as a menstruum to percolate the third portion of 400 grams of drug which had been moistened and packed under the same conditions as the portions above. This procedure was repeated with each successive portion of drug until, from the tenth and final percolator, containing ~~100~~ 50 grams of drug, only 50 cc of percolate were collected. The rate of flow in each percolator was so controlled that each sample of percolate was collected at approximately the same rate.

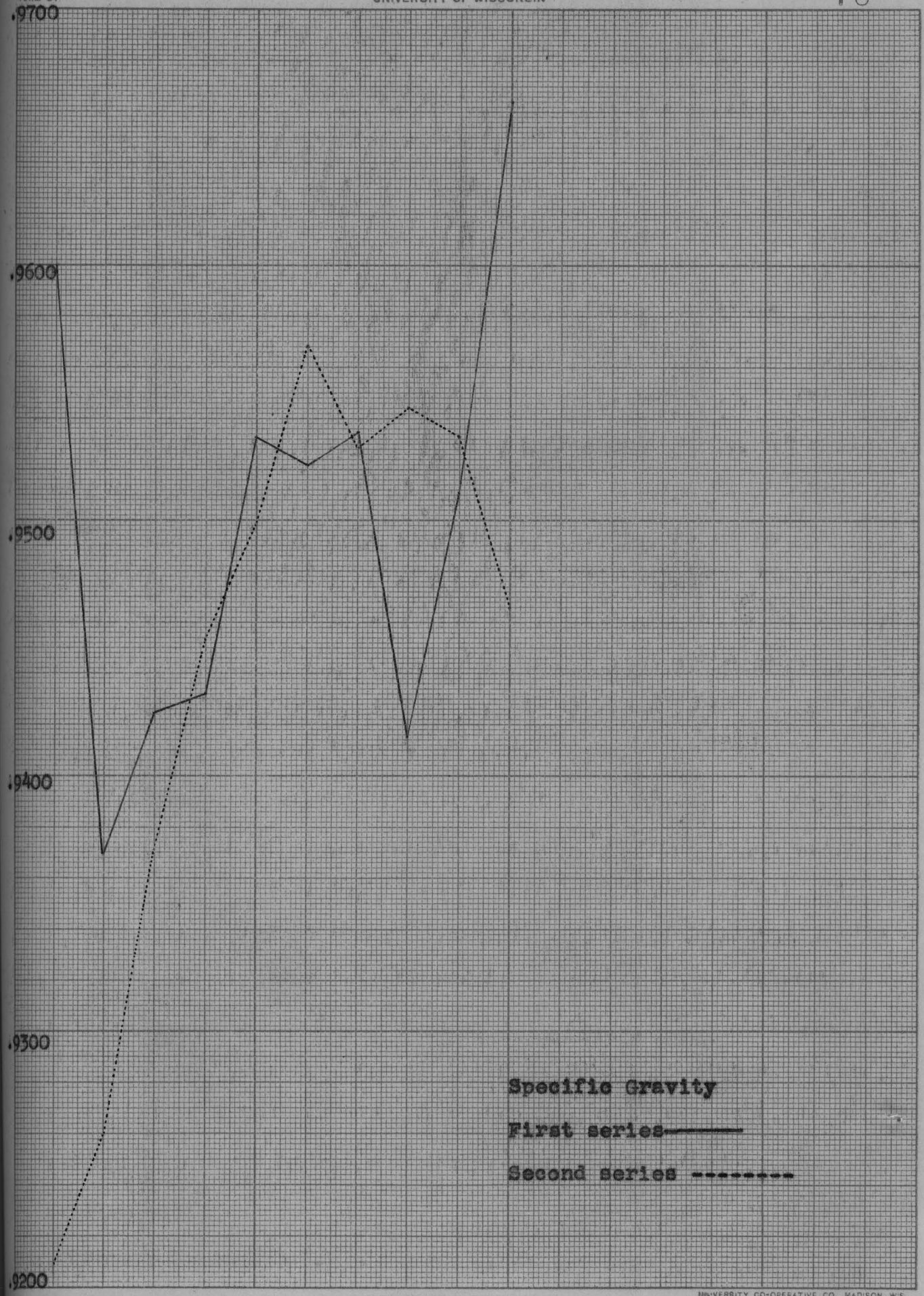
The ten samples of percolate of 50 cc each which were reserved above were used to determine specific gravity, total extractive, and tannin content. Twenty five cc were used in each case to determine the specific gravity by means of a pycnometer at 25°, 10 cc were used to determine total extractive by the U.S.P.X. method,[3] and 10 cc were used to determine the tannin content by the permanganate method.[4] The results obtained from two series of experiments are tabulated on the following page. Following the tabulation, the same data for the two series are presented graphically in order to show a clearer picture of the results and the irregularities obtained.

First series

Amount of Drug in Gms.	Rate in hours	Sp.Gr. at 25°	Total Wt.in Gms.	Extractive Percent	Tannin Wt. in Gms.	Content Percent
500	29	0.9600	2.3806	24.80	1.2418	12.93
450	27	0.9369	2.0908	22.32	1.3339	14.24
400	24	0.9425	2.2379	23.74	1.1424	12.12
350	21	0.9432	2.3321	24.72	1.8383	19.59
300	19	0.9533	2.4242	25.43	1.6860	17.68
250	17	0.9522	2.4406	25.63	1.5855	16.65
200	14	0.9535	2.3971	25.14	1.6961	17.79
150	11	0.9415	2.3188	24.63	2.1615	22.95
100	8	0.9511	2.5897	27.23	1.7096	17.97
50	5	0.9664	2.9238	30.25	1.6771	17.36

Second series

500	29	0.9209	2.0096	21.83	0.7877	8.55
450	26	0.9260	2.0357	21.98	1.0055	10.86
400	23	0.9371	2.1783	23.24	1.0745	11.46
350	20	0.9454	2.4069	25.45	1.1471	12.13
300	18	0.9499	2.5543	26.89	1.2306	12.95
250	15	0.9569	2.6929	28.14	1.1725	12.25
200	12	0.9529	2.5567	26.83	1.0926	11.47
150	9	0.9544	2.5369	26.58	1.2451	12.94
100	6	0.9533	2.5339	26.58	1.2269	12.87
50	3	0.9466	2.4419	25.79	0.9983	10.55



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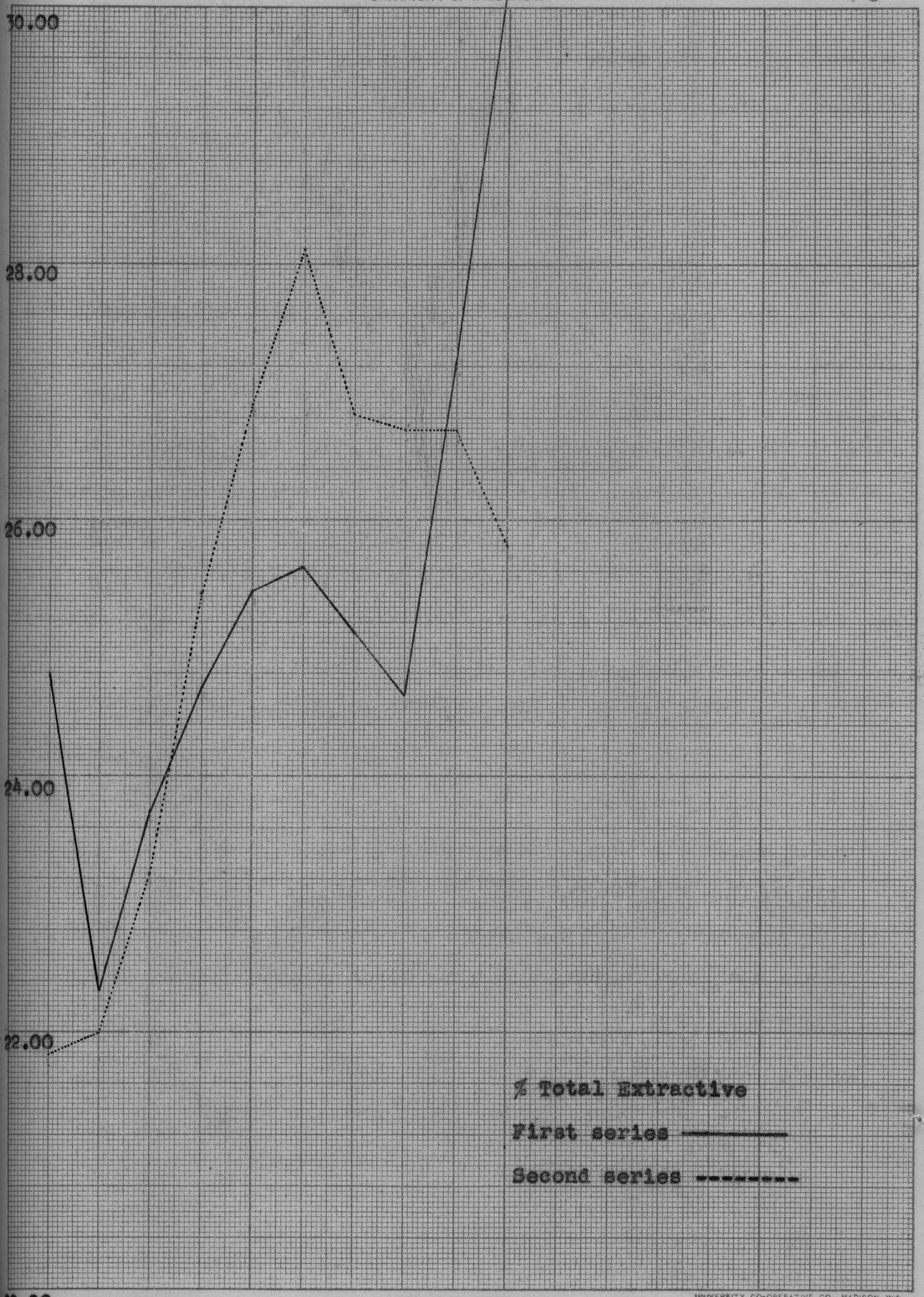

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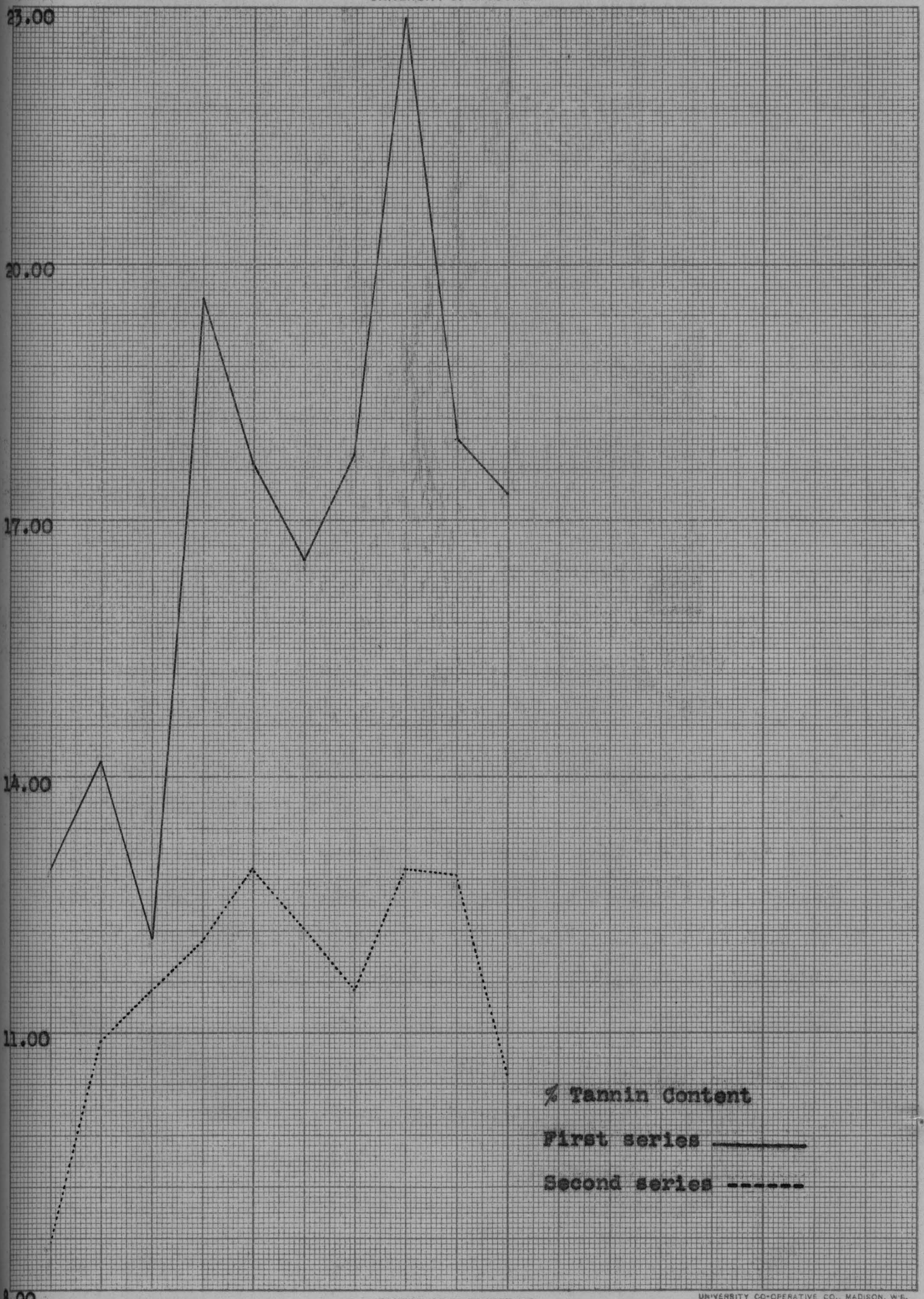

23.00
20.00
17.00
14.00
11.00
8.00
% Tannin Content
First series
Second series

Discussion

It has been assumed, perhaps logically, that during the extraction of a drug by percolation the percolate becomes more and more concentrated as the menstruum passes through the drug, until, when it reaches the bottom of the percolator, it is at the most concentrated stage. That this assumption does not hold true in the percolation of cinchona with alcohol was shown by Wruble.[1] His results and also those of Powers[2] show that a percolate in passing through a column of cinchona fluctuates in strength, and that the specific gravity, total extractive, and alkaloidal content reach a maximum point in each case which is followed by a decrease.

The anomalies observed by Wruble and Powers in the percolation of cinchona have also been observed in the percolation of Uva Ursi. In the second series of experiments presented graphically above it will be noted that the specific gravity, total extractive, and tannin content reached a maximum point which was followed by a decrease.

The results of the first series of experiments are presented but form no basis for comment and should probably be disregarded or held in abeyance until more data is obtained. It is believed that the inconclusive results obtained in this series were due to an unfamiliarity with the details of the technique required, especially so in the case of the tannin assay, which offers many sources of error even in the hands of an experienced operator.

Wruble, in commenting upon his results, stated that

they were not readily explainable, but suggested that the little understood phenomena of adsorption, absorption, and other surface phenomena were perhaps responsible in a large measure for the anomalies observed by him. No attempt is made to explain the results obtained here in the experiments on Uva Ursi. They are presented as a further confirmation of the results of Wruble and Powers with the hope that at some future time, when sufficient data of this sort have been obtained from other sources and with other drugs, they may be helpful in formulating a better understanding of the whole problem of drug extraction.

1). Wruble,M.,J.Am.Pharm.Assoc.22,641,1933.

2). Powers,J.L.,Pharmaceutical Archives 7,65,1936.

3). U.S.Pharmacopoeia,10th Revision,p.466.

4). Villavecchia,V.,Applied Analytical Chemistry,Ed.2,337,1918.

Ursone (Ursolic Acid) and Isomeric Compounds

In 1854 H. Trommsdorff[1] isolated a crystalline compound from the leaves of Arctostaphylos uva-ursi which he named urson. Hlasiwetz,[2] in 1855, assigned to it the formula $(C_{10}H_{17}O)_n$. Rochleder,[3] in 1866, isolated from the leaves of Epacris a substance having the properties of Trommsdorff's urson and which on analysis gave the composition $(C_{10}H_8O)_n$. In 1893 Gintl[4] made the first thorough investigation of a product prepared by Merck and assigned to it the formula $C_{30}H_{48}O_3$. Next Dodge,[5] in 1918, made a critical comparison of urson and caryophyllin, regarding both as isomeric lactones. Nooyen,[6] in 1920, used the method of Dodge to investigate the presence of urson in a number of plants. By applying the Liebermann color test as a positive test for urson in the product isolated, she found urson to occur in twenty ericaceous plants and in a number of the Ilex family. Van Itallie,[11] in 1921, found urson in the mistletoe. In 1923 Sando[7] isolated malol from apple peelings and pointed out that the oleanol of Power and Tutin[8] (1908), further investigated by Tutin and Naunton[10] (1913), also the prunol of Power and Moore[9] (1910) are closely related compounds.

Since then ursone and related compounds have been investigated by the following:

Van der Haar (1922)
" " " (1924) (4 references)
" " " (1927)
" " " (1928)
Ruzicka and Van Veen (1929) (2 references)
Ruzicka and coworkers (1931-1936) (series of numerous papers)

Dodge (1930)

Sando (1931)

Jacobs and Fleck (1931)

" " " (1932)

Winterstein and Stein (1931) (8 references)

" " " (1932) (2 references)

" " " (1933)

Winterstein and Hammerle (1931)

Wedekind and coworkers (1931-1936) (series of papers)

Kitasato and coworkers (1932-1936) (series of papers)

Kuwada and Matsumato (1933)

Kuwada and Matsukawa (1933) (3 references)

Kuwada and Matsukawa (1934) (2 references)

Cohee and St. John (1934)

Markley, Hendricks, and Sando (1935)

Sell and Kremers (1935)

Sando, Markley, and Matlack (1936)

Drake and Duvall (1936)

Markley and Sando (1937)

Yet in spite of these numerous efforts, the constitution of ursone and its relatives has not been determined. What is more, the structural type is still a muted question. Aside from the type groups, the rest of the molecule has been related to sesquiterpenes on the one hand[4)] and to sapogenins on the other.[12)] In addition, as early as 1893 Gintl[4)] showed that it gave the Liebermann-Salkowski color reaction for cholesterol.

Nomenclature. The German name, urson, as first given the compound by Trommsdorff, has been used interchangeably with the English spelling, ursone. Van der Haar,[13] in 1924, suggested the name, ursolic acid to conform with the acid character of the compound. This name has been adopted by all of the various investigators since that time.

Occurrence. As already pointed out, Trommsdorff,[1] in 1854, isolated ursone from the leaves of Arctostaphylos uva-ursi and Rochleder[2] in 1866, reported its isolation from an Epacris species. In 1920 Nooyen found it to be a constituent of as many as twenty (20) ericaceous plants. This might have lead to the assumption that ursone was a typical product of plants belonging to the family Ericaceae. However, she also found it in the Ilex family. Moreover, two years previously, Dodge[5] had pointed out the similarity between ursone and caryophyllin, regarding the two substances as isomers. Van Itallie,[11] in 1921, reported its isolation from the mistletoe, Viscum album. This was shown later by Winterstein and Stein[14] to be, not ursolic acid, but oleanolic acid, a closely related compound. Then, in 1923, Sando,[7] who had isolated malol from apple peelings, pointed out its similarity with oleanol, isolated from olive leaves by Power and Tutin[8] in 1908, and with prunol, isolated by Power and Moore[9] from the leaves of Prunus serotina in 1910. This similarity was further confirmed by Van der Haar when he showed that malol and prunol were identical with ursolic acid,[13) 15] and that oleanol, or oleanolic acid according to his new nomenclature, was isomeric with ursolic acid.[16]

In recent years ursolic acid has been isolated from the leaves of Arbutus Unedo by Sanna,[17] and from the leaves of

Rhododendron hymenanthes by Kuwada and Matsukawa.[18] Finally, Sando and his co-workers have found ursolic acid in the wax-like coating of the pear, Pyrus communis,[19] in the flowers and bracts of the flowering dogwood, Cornus Florida,[20] and in the cuticle on the skins of the cherry, Prunus Avium.[21]

It thus becomes apparent that "ursone" is not a natural product typical of a single family, but apparently is widely distributed. The following table contains the record of its occurrences observed thus far arranged according to the Engler and Prantl system. For the convenience of those who desire to acquaint themselves with other constituents of the species here under consideration, the numbers used by Wehmer in his "Die Pflanzenstoffe" are given in parenthesis. Additional sources to those given above are listed as they are cited in Wehmer with the reference given.

Family-Ericaceae

Number in Wehmer	Genus and Species	Reference
(3086)	Arctostaphylos uva ursi (L) Sprengl.	
(3066)	Rhododendron maximum L.	-
(3070)	Rhododendron hybridum L(?)	6)
(3063)	Rhododendron ferrugineum L.	6)
(963)*	Rhododendron hymenanthes Mak.	18)
(3088)	Vaccinium Myrtillus L.	6)
(3089)	Vaccinium Vitis-Idaea L.	6)
(3090)	Vaccinium uliginosum L.	6)
(3091)	Vaccinium Macrocarpum Ait.	6)
(3092)	Vaccinium oxycoccus L.	6)
(3100)	Erica Tetralix L.	6)
(3101)	Erica arborea L.	6)
(117)*	Arbutus Unedo L.	17)
-	Arbutus Andrachne -	6)
-	Azalea amoena _.	6)
-	Azalea indica __.	6)
-	Azalea sinensis __.	6)
(3058)	Ledum palustre L.	6)
(3077)	Kalmia angustifolia L.	6)
-	Andromeda japonica __.	6)
(3073)	Epigaea repens L.	22)
(3081)	Gaultheria procumbens L.	22)

* These numbers are found in the 1935 supplement of Wehmer.

Family-Aquifoliaceae

Number in Wehmer	Genus and Species	Reference
(2312)	Ilex Aquifolium L.	6)
(2318)	Ilex paraguariensis St.Hil.	6)
-	Ilex crenata __.	6)
-	Ilex perado __.	6)

Family-Rosaceae
Sub-family-Prunoideae

(1507)	Prunus serotina Ehrh. (Prunus virginiana) Mill.	9)
(1563)	Prunus avium L.	21)

Family-Rosaceae
Sub-family-Pomoideae

(1508)	Pyrus communis L.	19)
(1507)	Pyrus Malus L.	7)

Family-Empetraceae

(2260)	Empetrum nigrum L.	23)

Family-Cornaceae

(3038)	Cornus Florida L.	20)

Family-Pirolaceae

(3057)	Pirola medica Sw.	24)
(3053)	Chimaphila umbellata (L) Nutt.	24)

Family-Epacridaceae

(3103)	Epacris species ?	2)

Structure of the "Ursone" molecule. In spite of the large amount of experimentation with "ursone" and related compounds that has been going on for more than a decade, the structure of this substance has not yet been revealed. What is more, the seemingly simple problem of whether it is a hydroxy acid or a lactone appears still to be a muted question. As to the body of the molecule, the Liebermann-Salkowski reaction applied by Gintl[4] in 1893, seemingly at least, related it to cholesterol. His reduction with HI and phosphorus, also the distillation with zinc dust, yielding a sesquiterpene, indicated resemblance to polyterpenes. Finally, Ruzicka[13] regarded it as related to sapogenins. While all three viewpoints are suggestive to the modern structural chemist, they do not enable the interpreter of the experimental data to formulate a satisfactory picture of the structure of the molecule.

Elementary analysis and molecular weight determination- One of the most fundamental procedures in the characterization of an organic compound, i.e., the calculation of its molecular formula, has proved to be the most troublesome single problem in the work upon ursolic acid. Not only have the different investigators disagreed with one another in establishing the carbon and hydrogen content of the molecule, but, more unfortunately, no means is as yet at hand for obtaining such data with the degree of accuracy that is required in this instance. Two formulas have been put forth by the various workers, the first being $C_{30}H_{48}O_3$, and the second $C_{31}H_{50}O_3$. When it is shown that the

first formula requires a percentage composition of C-78.88; H-10.60, while the second requires C-79.08; H-10.71, an insight into the difficulty may be gained. The differences of 0.20 percent in carbon content and 0.11 percent in hydrogen content between the two formulas are within the limits of error that could be expected for results obtained by combustion analysis.

Contrary to a statement in the literature,[5] Trommsdorff did not assign a formula to his urson. A study of the original literature shows that Hlasiwetz[2] made an elementary analysis of a sample of urson obtained from Trommsdorff and assigned to it the formula, $(C_{10}H_{17}O)_n$. Gintl,[4] in 1893, was the first to assign to urson the molecular formula, $C_{30}H_{48}O_3$. Since that time many elementary analyses have been reported. Nooyen, in reporting the results of analyses of samples of urson obtained from six different plants, found her results to agree with those of Gintl for the same formula, while Sando,[7] in reporting the isolation of malol, later shown to be ursolic acid, assigned to it the formula, $C_{30}H_{48}O_3$.

It was Power and Moore,[9] in 1910, who first assigned the formula, $C_{31}H_{50}O_3$, to their newly isolated "prunol", later shown to be ursolic acid. Their formula was further substantiated by Van der Haar[25] from the results of his analysis. Van der Haar, in presenting his results, pointed out that those of Sando were open to error because they were obtained by macro-combustions. Sando, in his reinvestigation of ursolic acid in 1931,[26] responded with the results from ninety-two carefully controlled combustion analyses of ursolic acid and its derivatives.

To the impartial observer it is hard to make a choice between

the two formulas in question. It may be pointed out that the compound analysed by Van der Haar had a melting point of 279-80°, while that of Sando melted at 284-5°, showing that the question of purity may have influenced the results obtained. On the other hand, however, the criticism of macro-combustion of such high molecular compounds which was made by Van der Haar seems to be a just one which is held by other investigators.[32] However, the question of the molecular formula of ursolic acid, on the basis of evidence presented thus far, should still be regarded as an open one. Winterstein and Stein,[27] in 1931, carried out both combustion analyses and titration with standard alkali upon ursolic acid and its derivatives. They observed that the results of their combustions agreed with the $C_{30}H_{48}O_3$ formula, while titration values agreed almost equally as well for the $C_{31}H_{50}O_3$ formula. These authors pointed out that a choice between the two formulas is important from the phytochemical standpoint in gaining an insight into the formation of ursolic acid and its relation to other closely related plant constituents. By assuming for it the formula, $C_{30}H_{48}O_3$, ursolic acid could be conceived as an oxidation product of a triterpene alcohol, $C_{30}H_{49}OH$, such as the amyrins, while with the formula, $C_{31}H_{50}O_3$, the assumption might be made that the triterpene alcohols are formed through decarboxylation of the corresponding carboxy acids.

In the following table is given a summary of the results obtained by various investigators from the combustion analysis of ursolic acid. Results of the analysis of the numerous derivatives of the compound would require too much space to be given here and are no more enlightening in the problem at hand. These results may be found by referring to the original literature which is cited.

Carbon and Hydrogen Results Obtained by Various Investigators

bstance	Source	Investigator	No. of Combustions Reported	Mean Value for C	Mean Value for H
rson	Arctostaphylos uva ursi	Gintl, 1893 [4]	3	78.73	10.97
rmol	Prunus serotina	Power and Moore, 1910 [9]	1	78.71	10.98
rson	"Merck"	Van Itallie, 1918 [23]	1	79.07	10.55
"	Calluna vulgaris	Nooyen, 1920 [6]	2	78.78	10.33
"	Erica tetralix	"	2	78.84	10.46
"	Vaccinium macrocarpum	"	1	78.80	10.51
"	Ilex aquifolium	"	2	78.87	10.48
"	Empetrum nigrum	"	1	78.85	10.55
lol	Pyrus malus	Sando, 1923 [7]	2	78.75	10.54
rson	"Merck"	Van der Haar, 1924 [25]	2	79.31	10.93
lol	Pyrus malus	Sando, 1931 [26]	9	78.79	10.58
"	" "	"	9	78.82	10.58
rmol	Prunus serotina	"	10	78.79	10.67
rsolic Acid	Arctostaphylos uva ursi	"	10	78.77	10.56
"	"Schuchardt"	Winterstein and Stein, 1931 [27]	4	78.97	10.60
"	Arctostaphylos uva ursi	Jacobs and Fleck, 1931 [28]	2	78.78	10.78
"	" "	Kuwada and Matsukawa, 1933 [19]	1	78.75	10.64
"	Pyrus communis	Sando et al, 1935 [20]	1	78.59	10.78
"	Cornus Florida	" 1936 [29]	1	79.02	10.72
"	A. uva ursi	Drake and Duvall, 1936 [29]	2	78.95	10.61
"	Prunus avium	Sando et al, 1937 [21]	1	78.74	10.67

Molecular weight determinations have been reported by a few of the investigators, but here again the same difficulty has been encountered which was met with in the combustion analyses, viz., the methods used do not have sufficient accuracy to differentiate between a molecular weight of 456 for the formula, $C_{30}H_{48}O_3$, and a molecular weight of 470 for the formula, $C_{31}H_{50}O_3$. Such estimations as have been made have followed along one of three methods, namely physical methods, alkali titration, and analysis of metallic salts of the ursolic acid.

Physical methods- Gintl[4] made the first molceular weight determination on ursolic acid. By the cryoscopic method, using phenol as a solvent in an Eykmann Depressimeter, he obtained molecular weights ranging from 446 to 515. Nooyen,[6] using the cryoscopic method, arrived at a mean molecular weight of 456, while Kuwada and Matsukawa,[30] using the same method, obtained a molecular weight of 487.

Alkali titration- By titration with standard potassium hydroxide Dodge[5] obtained an acid value of 122.7 for ursolic acid, corresponding to the formula, $C_{30}H_{48}O_3$, which, assuming it to contain one COOH group, would have a theoretical acid value of 122.9. The formula, $C_{31}H_{50}O_3$, would have a theoretical acid value of 119.1. Nooyen[6] found acid values agreeing with the results of her cryoscopic method for the formula, $C_{30}H_{48}O_3$. Winterstein and Stein,[27] on the basis of four titrations, found an average molecular weight of 463.

Analysis of metallic salts- Sando[7] found the sodium salt of ursolic acid to contain 4.66 percent of Na, while Power and

Moore[9] found it to contain 4.6 percent. $C_{30}H_{47}O_3Na$ requires 4.81 percent of Na, while $C_{31}H_{49}O_3Na$ requires 4.67 percent. Sell and Kremers[31] reported the preparation and analysis of twelve metallic salts of ursolic acid. Although they did not have in view the use of their results as an aid in estimating the molecular weight of the compound, their results may be cited here in that connection. In presenting their analyses these authors calculated the theoretical content of the metallic element on the basis of the formula, $C_{30}H_{48}O_3$. In order to make the picture more complete another column has been added to the following table of their results. This added column shows the theoretical content of the metallic element calculated on the basis of the $C_{31}H_{50}O_3$ formula.

Salt	Metal determined as	Metal found(%)	Metal Calc. on basis of $C_{30}H_{48}O_3$	Metal Calc. on basis of $C_{31}H_{50}O_3$
Aluminum ursolate	Al_2O_3	2.0	1.94	1.88
Barium ursolate	$BaSO_4$	12.8	13.12	12.77
Calcium "	$CaSO_4$	4.0	4.21	4.09
Cadmium "	$CdSO_4$	11.1	10.99	10.70
Cobalt "	$CoSO_4$	5.9	6.08	5.91
Lead "	$PbSO_4$	18.5	18.54	18.09
Lithium "	Li_2SO_4	1.3	1.50	1.45
Magnesium "	$MgSO_4$	2.4	2.60	2.52
Manganese "	$MnSO_4$	5.3	5.69	5.53
Nickel "	$NiSO_4$	5.4	6.06	5.89
Silver "	Ag	18.7	19.16	18.70
Strontium "	$SrSO_4$	8.2	8.77	8.54

Functional groups in the "Ursone" molecule- As already pointed out, Dodge,[5] as early as 1918, suggested a lactone structure; to be more specific, a hydroxy lactone structure, $C_{29}H_{47}\begin{matrix}OH\\ \diagup\\ O\\ \diagdown\\ CO\end{matrix}$. This was denied by Van der Haar,[25] in 1924, who claimed that it was a hydroxy acid, $(C_{15}H_{24})_2\begin{matrix}OH\\ \diagup\\ \_COOH\end{matrix}$.

The acid character, also the inner ester character, is indicated first of all by the formation of salts. Most of the salts reported for ursolic acid have been listed previously under the heading of molecular weight determinations. Perhaps the best way to take up the discussion of the functional groups in the molecule is to note briefly and in as near chronological order as feasible the evidence presented by each investigator.

Gintl[4] reported the absence of carbonyl and methoxyl groups but presented evidence of a hydroxyl group in the form of acetyl and benzoyl derivatives. He obtained no evidence for a carboxyl group. Dodge[5] reported the preparation of potassium, magnesium, and lead salts, and offered as evidence of a lactone structure the stability of the compound toward heat, the tendency of its salts to hydrolyse, the decomposition of its salts by carbon dioxide, and the preparation of a diacetate, which, upon heating, lost acetic acid to form a monoacetate. It is to be noted that Dodge was aware of the fact that the instantaneous neutralisation of the compound in alcoholic solution by alcoholic alkali did not argue well for a lactone structure. Nooyen[6] prepared a crystalline potassium salt as well as the sodium, silver, lead, calcium, and barium salts in amorphous state. She also reported the absence of aldehyde, ketone, and methoxyl groups, and, contrary to Gintl, could prepare no acetyl or

benzoyl derivatives. Evidence of a carboxyl group was obtained in the form of the salts and a methyl ester.

Power and Moore[9] reported a diacetyl derivative of prunol which, upon boiling with alcohol, lost one acetyl group and yielded a monoacetyl derivative. They were of the opinion that two hydroxy groups, one phenolic and the other alcoholic in nature, were present, but did not explain the structural significance of the third oxygen atom. They apparently regarded as structurally insignificant the fact that they were able to prepare a sodium salt as well as a methyl derivative. Again, Sando[7] regarded malol as a "crystalline alcohol" and made no mention of the presence of a carboxyl group, although he reported a sodium salt as well as a methyl derivative. In a similar way to that of Power and Moore, Sando prepared a diacetyl derivative which, upon boiling with alcohol, lost one acetyl group and yielded a monoacetyl derivative. He also reported an acetyl methyl derivative.

It was Van der Haar[25] who first reported the ursolic acid molecule to contain an alcoholic OH and a COOH group and who regarded it as a monohydroxy-monocarboxy acid. He discredited Dodge's view of a hydroxy lactone structure on the basis of his own observation of the evolution of CO_2 upon zinc dust distillation, and also pointed out that the instantaneous neutralization of the compound by alkali and the opening of a lactone ring upon acetylation are reactions not generally exhibited by the commonly known lactones. By the Tschugaeff-Zerewitinoff method Van der Haar found two "active" hydrogens, corresponding to two OH groups in ursolic acid. He reasoned, logically, that if the

hydroxy lactone structure were assumed to be the correct one, then by the formation of a salt and from this salt an ester, there must be gained an additional OH group. By the above method, however, he found only one OH group in methyl ursolate prepared in this way, and confirmed this evidence by the further observation that the methyl ursolate yielded only a monoacetyl derivative and not a diacetyl derivative. The fact that Van der Haar did not confirm the observations of Sando and others as to the formation of a diacetyl derivative of ursolic acid will be referred to later.

Sando,[26] in 1931, provided further evidence against the hydroxy lactone structure and in favor of the hydroxy acid structure. Starting with monoacetyl ursolic acid he prepared, by means of thionyl chloride, the acid chloride (monoacetyl ursolyl chloride) which he converted into the corresponding methyl ester (monoacetylmethyl ursolate) by boiling with methyl alcohol. This ester was identical with one prepared by methylating ursolic acid with sodium ethoxide and methyl iodide, and following this treatment by acetylation. Sando reasoned that the formation of a methyl ester on boiling the product formed by thionyl chloride with methyl alcohol can only take place by assuming that the thionyl chloride reacted with a COOH group to form the acid chloride.

Thus it may be seen that the preponderance of evidence favors the hydroxy acid structure for the ursolic acid molecule. Suffice it to say, this conception has remained unchallenged since 1931.

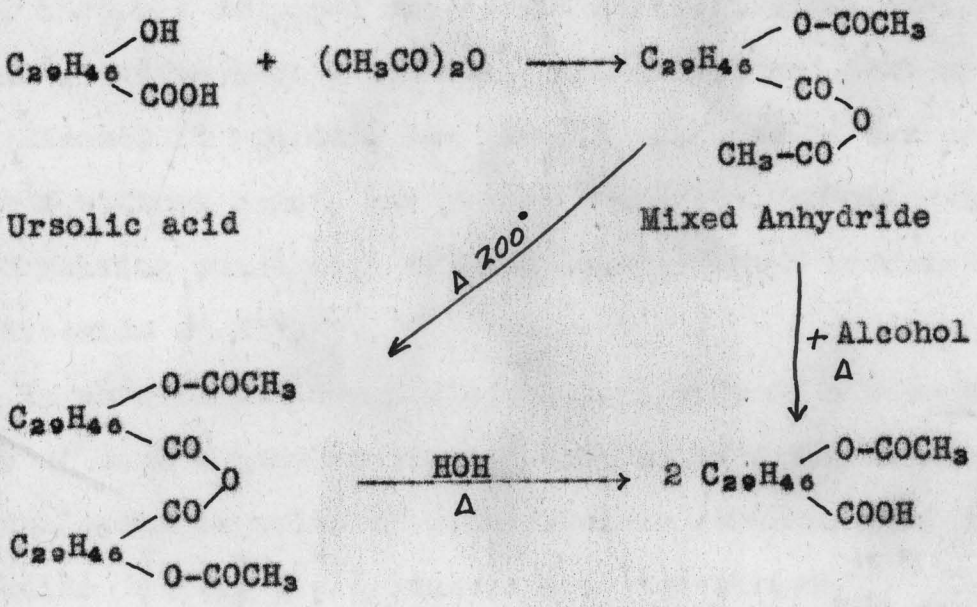
The question of a diacetyl derivative of ursolic acid- As mentioned previously, Dodge[5] observed that the diacetyl derivative of ursolic acid upon heating to 135° gave off acetic acid to form the monoacetate, which latter then melted at about 165°. This observation was repeated by Power and Moore,[9] who found that diacetyl prunol upon boiling with alcohol lost one acetyl group and yielded monoacetyl prunol. They found also that diacetyl prunol melted at 181°, but upon raising the temperature to 220° acetic anhydride was evolved, after which the mass resolidified and melted again only above 300°. The high melting compound thus formed analysed for $C_{66}H_{102}O_7$. Power and Moore expressed their hypothesis of this change as follows:

$$2\ (CH_3COO)_2C_{31}H_{48}O \longrightarrow (CH_3COO)_2O + \begin{matrix} CH_3COOC_{31}H_{48}\text{-}O \\ CH_3COOC_{31}H_{48}\text{-}O \end{matrix}\!\!>\!O$$

They offered no experimental evidence in support of their hypothesis other than an analysis of the resulting compound. Sando[7] confirmed the observations of Power and Moore in his experience with diacetyl malol, the latter melting at 199-200° with the evolution of gas, after which it resolidified and did not melt again under 300°.

Riviere and Pichard[33] claimed that the diacetyl derivative reported by Sando was not a true diacetyl derivative but rather a mixed anhydride of monoacetyl ursolic acid and acetic acid, which decomposed upon heating at 200° to form two symmetrical anhydrides, namely, acetic anhydride and monoacetyl ursolic acid anhydride, the latter melting above 300°. They further believed that the ursolic acid anhydride thus obtained

yielded, upon boiling with water, the same monoacetyl ursolic acid as did the mixed anhydride upon boiling with alcohol. Expressed in the form of equations their hypothesis was as follows:

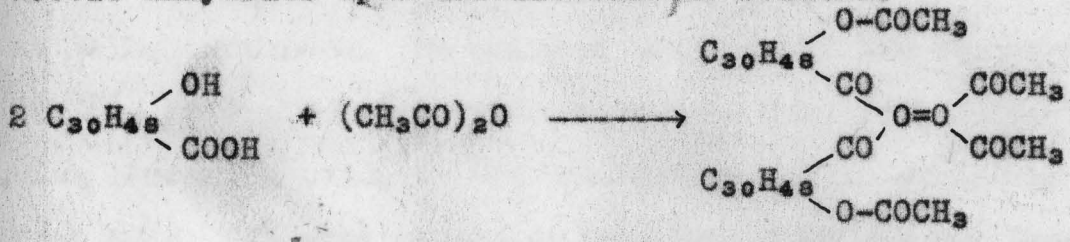


These authors, however, failed to support their hypothesis with enough conclusive evidence.

Van der Haar,[25] in 1924, pointed out that since ursolic acid contains only three oxygen atoms in all and since two of them were taken up in the COOH structure, a diacetyl derivative was impossible because only one oxygen was left to be accounted for in the form of an OH group. He reported a monoacetyl derivative melting at 200-01° (the same M.P. as Sando's diacetyl derivative), which, upon boiling with alcohol, yielded a crystalline compound, M.P. 276°. Van der Haar assumed, without evidence, this latter compound to be regenerated ursolic acid, the acetyl group having been removed by the action of the alcohol. Later, however, in 1928 Van der Haar[34] recognized

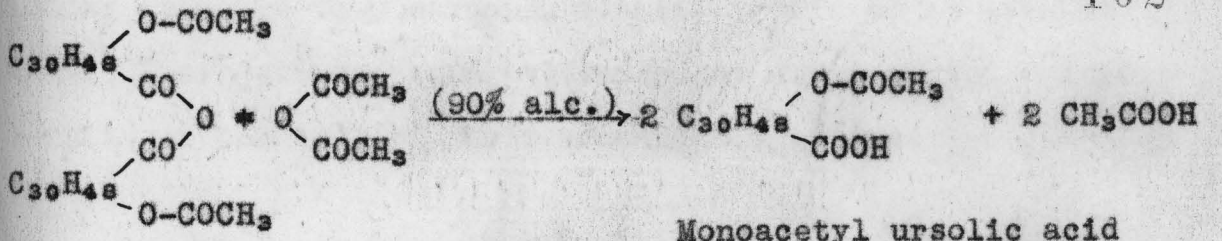
the error in his first observations and accepted the claims of Dodge, Sando, and others that a diacetyl derivative of ursolic acid could be obtained. He pointed out, after more painstaking work, that his supposed monoacetyl derivative, melting at 200-01°, was in fact a diacetyl derivative, and that upon boiling with alcohol it yielded, not ursolic acid, as he had previously assumed without proof, but rather monoacetyl ursolic acid. A mixed melting point test of this compound with ursolic acid gave a depression of 30°.

In an attempt to explain the formation of a diacetyl derivative of such a monohydroxy-monocarboxy acid Van der Haar cited the analogous behavior of other similar structures of a lower molecular weight, i.e., benzoic acid and others.[35] According to these analogies and his new data he explained the action of acetic anhydride upon ursolic acid as follows:



Diacetyl ursolic acid anhydride with one mole of acetic anhydride of crystallisation. M.P. 200-01°.

This compound melted at 200-01° with loss of acetic anhydride. Upon raising the temperature it resolidified and melted again at 320-22°, the melting point of the true anhydrous diacetyl ursolic acid anhydride. The action of boiling alcohol upon this compound he explained as follows:



Monoacetyl ursolic acid

As experimental evidence supporting these postulations Van der Haar observed that the compound melting at 200-01° and claimed by him to be diacetyl ursolic acid anhydride with a molecule of acetic anhydride of crystallisation, when dried to constant weight at 220° suffered a loss in weight corresponding to the theoretical loss in weight which would accompany the removal of one mole of acetic anhydride. The resulting product, the true diacetyl ursolic acid anhydride, gave a molecular weight corresponding to $C_{66}H_{102}O_7$, the formula assigned to it by Power and Moore.[9)]

Dodge,[36)] in 1930, commented on Van der Haar's conclusions as being anomalous. He pointed out that if the compound described by Van der Haar is a true acid anhydride, then boiling with alcohol should, by all analogy, yield an ester and not the free acid. It must be noted, however, that Van der Haar used alcohol containing some water (90%) and not absolute alcohol. Dodge's comments do not lessen the significance of the experimental evidence presented by Van der Haar, since he himself offered no further evidence. Sando,[26)] in 1931, made no comment on Van der Haar's explanation of the formation of a diacetyl derivative, thus apparently accepting it. In this connection, it should be pointed out that Kuwada and Matsukawa[30)] later reported a monoacetyl derivative melting at 295-6°, which, upon treatment with alkali, yielded a compound M.P. 288-90°; the

latter compound they assumed, without proof, to be ursolic acid. These authors were evidently not aware of Van der Haar's results at the time of their own investigations.

Nature of the hydroxyl group- Jacobs and Fleck[28] showed the hydroxyl group in ursolic acid to be apparently secondary in nature, when, by oxidation of the methyl ester with chromic acid in acetic acid solution they obtained a ketone, which they called ursonic acid methyl ester, M.P. 192-3°, and which yielded an oxime, M.P. 243-4°. Kuwada and Matsukawa[37] in 1933, oxidised ursolic acid itself with chromic acid in acetic acid solution and obtained a keto acid, ursonic acid, M.P. 284-5°; oxime, M.P. 274-6°; semicarbazone, M.P. 209°. By methylation of this keto acid with diazomethane they obtained the ursonic acid methyl ester, M.P. 193-5°, which formed an oxime, M.P. 241-2°, confirming the observations of Jacobs and Fleck. Again, Markley, Hendricks and Sando,[19] in 1935, by the same procedure obtained ursonic acid, M.P. 284-5°, which formed an oxime, M.P. 263-4°.

The methyl ester of ursolic acid- Sando and others reported a methyl ester melting at 170-2°. Jacobs and Fleck[28] reported the isolation of two substances when ursolic acid was methylated with diazomethane. These were separated by fractional crystallisation from acetone. The first, melting at 221-2°, gave only about one-half the theoretical methoxyl content for methyl ursolate and remained unchanged after boiling with alcoholic potassium hydroxide for four hours. The second, melting at 110-20°, corresponded in all respects to the monohydrate of ursolic acid

methyl ester previously described by Sando and others. Jacobs and Fleck explained their first product as a possible molecular association compound of ursolic acid methyl ester with some other substance of the same molecular order. Again, Kuwada and Matsukawa[30] reported two methyl esters; one, obtained with dimethyl sulfate, melting at 230° and giving only one-half the theoretical methoxyl content; the other, obtained with either diazomethane or methyl iodide and silver oxide, melting at 170-2°. Both of these substances upon acetylation yielded the same acetyl methyl ester and upon oxidation yielded the same ursonic acid methyl ester described above. In a later investigation Kremers, Sell, and Stookey[38] were able to show that one and the same methyl ester is formed by methylation of ursolic acid with either dimethyl sulfate, diazomethane, or methyl iodide.

Derivatives of ursolic acid- In order to summarise the derivatives of ursolic acid that have been prepared by the different investigators and which may or may not have been mentioned in the preceeding discussion, the following table is presented, giving the melting points of ursolic acid itself, its derivatives and reference to the investigator:

MELTING POINTS OF URSOLIC ACID AND ITS DERIVATIVES AS RECORDED IN THE LITERATURE

Ursolic Acid	Methyl Ester	* Mono-acetyl	* Diacetyl	Acetyl-methyl Ester	Benzoyl	Benzoyl-methyl Ester	Reference
264		264			214		4)
285		165					5)
272-4	148						6)
284-5	170.5	279-81	199	243-4			7)
275-7	164-5	290	181	235			9)
279-80	165	280-1	200-1				13)25)34)
284-5	171	289-90		246-7			26)
284-5	110-20					212-3 235-6	28)
284-5	170-2	288-90		246-7		217-8	30)
290-1	170-2			245-6		215-6	18)
278		264			214		17)
283-4	170-1						20)
281-2	168						21)
283-4	171	280		246			38)

* This column should be interpreted in the light of the discussion preceding.

In addition to these the following have also been reported:

Phenacyl, M.P. 199-200°[26]; M.P. 193°[38]

Phthalyl, M.P. 264-5°[27]

Phthalylmethyl ester, M.P. 214-5°[26]

Formyl ester, M.P. 258°[27]

p-phenylphenacyl, M.P. 207.5-09°[19]

Kremers, Sell, and Stookey[38] have also prepared the following:

Acetyl ursolyl chloride, M.P. 200.5°
Acetylethyl ursolate M.P. 246
Acetylpropyl ursolate M.P. 194
Acetyl n-butyl ursolate M.P. 125.5
Acetyl n-amyl ursolate M.P. 110.5
Acetyl n-hexyl ursolate M.P. 123.5
Acetyl n-heptyl ursolate M.P. 93
Acetyl n-octyl ursolate M.P. 67

Body of the "Ursone" molecule- If Dodge's lactone structure is assumed to be correct and if either formula, $C_{30}H_{48}O_3$ or $C_{31}H_{50}O_3$ is assumed to be the correct one, we would have $C_{29}H_{47}(OH)(CO)O$, or $C_{30}H_{49}(OH)(CO)O$. In either case the underlying hydrocarbon would be $C_{30}H_{52}$ or $C_{31}H_{54}$. Both of these hydrocarbons are representatives of the formula of saturation, C_nH_{2n-8}, under which are possible, barring triple bonds, the following configurations:

F_5

$F_4\,\Delta_1$

$F_3\,\Delta_2$

$F_2\,\Delta_3$

$F_1\,\Delta_4$

Δ_5

On the other hand, if Van der Haar's conception of a hydroxy acid is true, we have either $C_{29}H_{46}(OH)(COOH)$ or $C_{30}H_{48}(OH)(COOH)$. The underlying hydrocarbon in this case would be either $C_{30}H_{50}$ or $C_{31}H_{52}$. Both of these hydrocarbons are representatives of the formula of saturation, C_nH_{2n-10}, under which are possible, barring triple bonds, the following configurations:

F_6

$F_5\,\Delta_1$

$F_4\,\Delta_2$

$F_3\,\Delta_3$

$F_2\,\Delta_4$

$F_1\,\Delta_5$

Δ_6

Additive capacity- As may be seen from the possible configurations of the molecule listed above, the detection and

estimation of double bonds would aid greatly in determining which configuration is present. Gintl[4] reported that the action of bromine upon urson gave an amorphous body containing 60 percent of bromine. This apparently was a substitution product. Permanganate caused no oxidation. Nooyen[6] could detect no addition of bromine, iodine, ozone, or hydrogen, and obtained no oxidation with permanganate. Winterstein and Stein[27] observed, however, in 1931, that ursolic acid gave a yellow color with tetranitromethane, a color reaction used to detect ethylenic linkages which are too unreactive to be detected in any other manner.

The Position of "Ursone" Among Other Related Plant Constituents- As previously pointed out in this paper, the body of the ursolic acid molecule has been variously related to the sapogenins, sterols, and sesquiterpenes. In addition, it has been shown to have certain relationships to the resin acids and alcohols. Ruzicka has designated these various heterogeneous classes of natural products by the all-inclusive title of polyterpenes and polyterpenoids. Although the knowledge of even the more prominent members of these compounds is still incomplete in many respects, it has progressed far enough to show clearly that certain relationships do exist among them. In the remainder of this paper the evidence for the relationship of ursolic acid to other compounds in the category of polyterpenoids will be briefly cited.

Zinc dust distillation- Gintl[4] was able to show that urson

upon distillation with zinc dust yielded a hydrocarbon of the same composition as the sequiterpenes, $C_{15}H_{24}$, and which yielded a liquid bromide, a hydrochloride, and a nitrosate. Van der Haar,[39] in 1922, made a study of the zinc dust distillation of hederagenin, sitosterol, cholesterol, urson (ursolic acid), and oleanol (oleanolic acid), and showed that the hydrocarbons obtained in all cases were very similar with regard to their physical properties and reaction to the Liebermann color test. Again in 1924, Van der Haar[25] obtained a sesquiterpene by zinc dust distillation of urson. It may be noted, parenthetically, that he was also able to detect carbon dioxide, which influenced him to propose the formula, $C_{31}H_{50}O_3$, for urson and to explain its decomposition as follows:

$$C_{30}H_{48}(OH)COOH \longrightarrow 2\ C_{15}H_{24} + CO_2 + H_2O\ .$$

In connection with studies by zinc dust distillation Ruzicka has pointed out that the boiling points of the products obtained indicate a mixture of compounds of similar molecular weight rather than pure sesquiterpenes.

Dehydrogenation studies- Within the past few years a method which proved helpful in clearing up the structures of many of the sesquiterpenes, viz., dehydrogenation, has been applied with good success to many of the compounds described by Ruzicka under the title of polyterpenoids. Van der Haar,[25] by dehydrogenation with sulfur of both urson itself and the sesquiterpene noted above, was able to isolate a small amount of a hydrocarbon picrate melting at 115°, but which was not analysed. Ruzicka and Van Veen,[42] in 1929, included ursolic acid in a

list of eleven sapogenins which they dehydrogenated by means of selenium. From all of these they were able to isolate a hydrocarbon whose picrate melted at 127° and whose styphnate melted at 152°. These derivatives were found to be identical with the corresponding derivatives of sapotalene (1,2,7-trimethylnaphthalene), which had been obtained from the triterpene derivatives, amyrin, betulin, and lupeol. These authors pointed out that a close relationship must exist between the sapogenins and certain natural triterpenes, but that the carbon skeleton must differ essentially from that of the sterols since the latter do not yield a trimethylnaphthalene. Again in 1936, Drake and Duvall[29] obtained upon dehydrogenation of ursolic acid the sapotalene reported by Ruzicka and in addition 2,7-dimethylnaphthalene and a polymethyl picene, apparently identical with a substituted picene obtained by Ruzicka from related triterpenoid substances. The most fully characterized products of the dehydrogenation of these various triterpenoid substances are as follows:[43]

1,2,3,4-tetramethylbenzene
2,7-dimethylnaphthalene
1,2,7-trimethylnaphthalene (sapotalene)
1,2,7-trimethylnaphthol
1,2,5,6-tetramethylnaphthalene
a pentamethyl dinaphthyl, $C_{25}H_{24}$
a picene homologue, $C_{25}H_{20}$

The Unsaturated Cyclic Structure of the Polyterpenoids-

Although few attempts have been made to establish the cyclic structure and the position of unsaturation in ursolic acid itself, there has been an enormous amount of work done in this respect with the sapogenins and other triterpenoid substances. As a further effort in establishing the position of ursolic acid among these other natural products it is thought advisable to cite some of this work.

Catalytic hydrogenation- This method of detecting double bonds has not proved to be of great success in the case of the polyterpenoid substances. The double bond contained in these compounds is too unreactive to be detected in this manner.

Lactone formation- The relationship suspected by Van der Haar[39] between ursolic acid and the sapogenins prompted Winterstein and his coworkers to study their related aspects. These investigators have offered evidence[14) 40] showing the sapogenins, hederagenin and oleanolic acid, to be unsaturated pentacyclic acids. They were further able to show that hederagenin was converted by the action of bromine into a neutral, saturated bromo-lactone; also that by long heating with formic acid it was transformed into a neutral, saturated lactone, which lead them to assume a γ -, or δ - unsaturated pentacyclic acid structure for the compound. They found that oleanolic acid also yielded a neutral, saturated bromolactone and assumed an analogous structure for it. In their study of ursolic acid, however, they found, in contrast to the other sapogenins, that it reacted only very slowly and incompletely with bromine, yielding largely resinified products with a very small quantity of a neutral, bromine-containing body which

gave, in contrast to ursolic acid, no yellow color with tetranitromethane and which they assumed to be a bromo-lactone. In contrast to hederagenin the heating of ursolic acid with formic acid gave no lactone but only the normal formyl derivative. The action of ursolic acid upon fish, however, influenced them to place this compound in the class of sapogenins.

Molecular refraction- This method has been used to a limited extent in the detection and estimation of double bonds among the polyterpenoid substances. Ruzicka and coworkers,[44] by means of this method, reported the triterpene alcohols, α- and β- amyrins, $C_{30}H_{49}OH$, to contain one double bond.

Perbenzoic acid titration- Ruzicka and coworkers as well as many other investigators have applied this method quite successfully in estimating the number of double bonds in the polyterpenoid compounds. Winterstein and his coworkers have applied it especially to the detection of a double bond in hederagenin and oleanolic acid. They found that these compounds themselves did not react readily with the perbenzoic acid, but that their decarboxylated products reacted quite smoothly, showing the presence of one double bond.

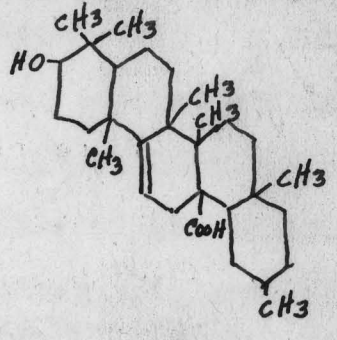
Decarboxylation and dehydration studies- By subjecting oleanolic acid, $C_{29}H_{46}(OH)COOH$, to the action of heat for one-half hour at 360-400°, Winterstein and Stein[45] were able to isolate the alcohol, oleanol, $C_{29}H_{47}OH$, resulting from its decarboxylation, and also the hydrocarbon, oleanylene, $C_{29}H_{46}$,

resulting from its decarboxylation and dehydration. Catalytic hydrogenation of this unsaturated hydrocarbon yielded the saturated pentacyclic hydrocarbon, oleanene, $C_{29}H_{48}$. By the same treatment of hederagenin,[46] $C_{29}H_{45}(OH)_2COOH$, and the boswellinic acids, $C_{30}H_{48}O_3$, from olibanum,[47] they obtained hydrocarbons which were isomeric but not identical with those obtained from oleanolic acid. They pointed out that the hydrocarbons obtained in these instances were not of the same series as those obtained from the amyrins, $C_{30}H_{49}OH$ by the same treatment.

Isomeric ursolic acids- Kuwada and Matsukawa[18] first introduced the possibility of there being more than one natural occurring ursolic acid when they isolated from Rhododendron hymenanthes a sample melting at 290-1°. They designated this as β-ursolic acid in contrast to α-ursolic acid, M.P. 284-5° which they isolated from uva ursi. In a later paper[49] they reported a spectrographic study of these two samples, along with hederagenin and oleanolic acid. They found that hederagenin, oleanolic acid, and α-ursolic acid showed the same general absorption curves, while the β-ursolic acid showed a different curve. From these results they suspected a stereoisomerism, lying in the -C-COOH linkage. The same authors investigated the possibility of the isomerism of oleanolic and ursolic acids due to the position of the OH group.[50] By treating the acids themselves with phosphorus pentachloride and reducing the resulting products with zinc and acetic acid they were able to prepare the corresponding desoxy-acids, in other words, to replace

the OH groups by hydrogen. The desoxy-acids thus prepared, however, were not identical, showing the cause of the isomerism not to be in the position of the OH group.

Summary- The compound, urson, (later called ursolic acid) first isolated from Arctostaphylos uva ursi by Trommsdorff in 1854 and since then found in several other plants has been characterized by fairly conclusive evidence as a monohydroxy-monocarboxy acid of the formula, $C_{30}H_{48}O_3$ or $C_{31}H_{50}O_3$. Most of the evidence favors the $C_{30}H_{48}O_3$ formula. It has been shown to have certain relationships in structure and properties to those plant products designated as sapogenins and to other triterpenoid substances, but it differs essentially in its structure from the sterols. Ursolic acid has been claimed by many investigators to be isomeric with the sapogenin, oleanolic acid. As a matter of interest the latest formula for oleanolic acid proposed by Ruzicka[48] is presented here:



Inspection of this formula shows it to be $C_{30}H_{48}O_3$, with the underlying hydrocarbon $C_{30}H_{50}$, a representative of the formula of saturation, C_nH_{2n-10}, containing one double bond and five cycles.

Decarboxylation of Ursolic Acid

Winterstein and Stein[45] have reported the decarboxylation of oleanolic acid, $C_{31}H_{50}O_3$, to the alcohol, oleanol, $C_{30}H_{49}OH$, dehydration of this alcohol to the unsaturated hydrocarbon, oleanylene, $C_{30}H_{48}$, and subsequent hydrogenation of this hydrocarbon to another hydrocarbon, oleanene, $C_{30}H_{50}$.

(a) The decarboxylation of ursolic acid was attempted by the method of Winterstein and Stein:

10 Gms. of ursolic acid were heated slowly in a 50 cc. round bottom flask until the acid had all melted, and then the temperature was raised to 360-400° and held there for 30 minutes. A funnel was placed in the neck of the flask to minimise loss due to sublimation. At the end of 30 minutes the mass was allowed to cool and solidify to a light brown brittle residue. This was taken up in ether and the ether solution shaken out repeatedly with saturated barium hydroxide solution to remove any unreacted ursolic acid. A considerable amount of gelatinous barium ursolate was separated from the ether solution in this manner. The ethereal filtrate was allowed to evaporate, leaving a semi-solid residue of light brown color. Attempts to obtain a crystalline product from this residue by recrystallisation from acetone, methanol, ethanol, and heptane, respectively, were unsuccessful. Winterstein and Stein reported the isolation of the alcohol, oleanol, and the hydrocarbon, oleanylene, from the decarboxylation of oleanolic acid at this point by fractional crystallisation from acetone. They also observed that a large portion of the decarboxylation products from oleanolic

acid were resinified. It was evident that with ursolic acid practically all of the decarboxylation products had become resinified. The entire resinous mass was then refluxed for two hours with acetic anhydride in the hope that, if any of the alcohol were present, it could be separated by means of its acetyl derivative. Upon taking up the acetylated product in ether a very few crystals were noted in the bottom of the container. The amount, however, was too small to allow any sort of separation.

(b) Use of a catalyst in decarboxylation:

It was thought that the use of a catalyst might accomplish the desired decarboxylation at a lower temperature than was necessary when using heat alone and thus avoid the large amount of resinous products that accompanied the decarboxylation with heat. Accordingly a quantity of ursolic acid was heated until it had melted and then copper chromium oxide catalyst was added and the mixture held at the melting point of ursolic acid for one hour. At the end of this time the mixture was allowed to cool and the solid, pulverised mass was extracted with ether. Investigation of the residue left upon evaporation of the ether showed it to consist almost entirely of unreacted ursolic acid accompanied by a very small amount of brown colored resinous products.

(c) Attempts at photochemical decarboxylation:

Saturated solutions of ursolic acid in ethanol and acetone were subjected to the effect of bright sunlight for

three days in the presence of ferric chloride and uranyl acetate, respectively. No CO_2 formation could be detected.

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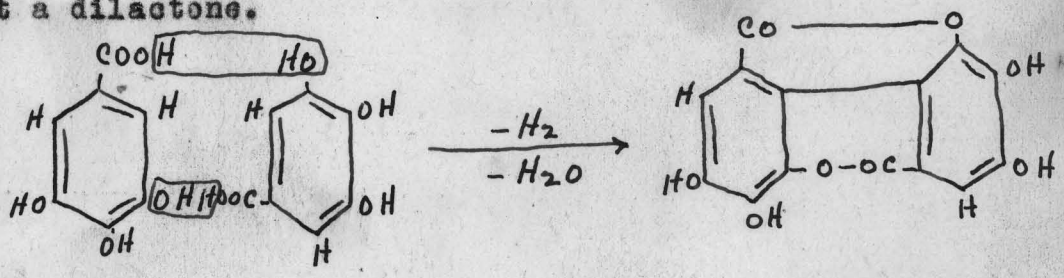
Ellagic Acid

History. When treating the insoluble residue, resulting in the preparation of gallic acid according to Scheele's method from an infusion of nutgalls, Chevreul[1] (1) in 1815 observed this substance which Braconnot in 1818 recognized as a peculiar acid to which he assigned the name ellag(ic) acid, the name resulting from the reverse spelling of Galle (French as well as German for nutgall) (2).

Its elementary composition was determined by Pelouze (3) who assigned to the anhydrous (?) acid the formula $C_7H_4O_4$+ or $C_7H_6O_5$, the same as that of gallic acid of which Berzelius regards it as a metamer.

The structural formula accepted at present is based on that first suggested by Schiff (4) in 1879 and proven by Graebe (5) in 1903. This formula brings out its relation to gallic acid, also the fact that the substance is not an acid but a dilactone.



Occurrence. Ellagic acid has been isolated from the following plant and animal sources (chronological record: Year, source, author's name, reference.)

1846.	Caesalpinia coriaria W.	Hamilton	Pharm. Journ. 5, 443 (1846)
1847.	Bezoar Stones	Wöhler and Merklein	Ann. Chem. Pharm. 55, 129 (1847)
1851.	Urinary Calculi	Goebel	Ann. Chem. Pharm. , 83 (1851)
1867.	Punica Granatum L.	Rembold	Ann. Chem. 143, 288 (1867)
1875.	Caesalpinia coriaria W.	Löwe	Zeit. Anal. Chem. 14, 40 (1875)
1875.	Terminalia Chebula R.	Löwe	Ibid. 14, 35 (1875)
1879.	Caesalpinia coriaria W.	Barth and Goldschmidt	Ber. 12, 1237 (1879)
1880.	Castanea Vesca G.	Steltzner	Am. J. Pharm. 52, 292 (1880)
1880.	Quercus Robur L.	Etti	Monatsh. Chem. 1, 226 (1880)
1881.	Quercus Robur L.	Strohmer	Monatsh. Chem. 2, 539 (1881)
1881.	Picea Excelsa Lk.	Böttinger	S. Ber. Wien. Acad. 84 (1881)
1882.	Nymphea alba L.	Grüning	Arch. Pharm. 20, 589 (1882)
1891.	Caesalpinia brevifolia Baill.	Zölffel	Arch. Pharm. 229, 123 (1891)
1891.	Terminalia Chebula R.	Zölffel	Ibid.
1896.	Quebracho Colorado	Perkin and Gunnell	J. Chem. Soc. 69, 1307 (1896)
1897.	Haemotoxylon Campeachiamum L.	Perkin	Ibid. 71, 1137 (1897)
1897.	Quercus lusitanica Lam.	Perkin	Ibid. 71, 1131 (1897)
1898.	Tamarix Africana Poir.	Perkin	Ibid. 73, 374 (1898)

1900.	Arctostaphylos Uva Ursi (L) Spreng.	Perkin	Ibid.77,424(1900)
1900.	Coriara myrtifolia L.	Perkin	Ibid.77,424(1900)
1900.	Polygonum Bistorta L.	Bjalobrsheski	Pharm.Journ.(Russ.) 32,3(1900)
1903.	Vaccinium Vitis_Idaea L.	Kanger	Arch.Exper.Pathol.u. Pharmak.50,46(1903)
1905.	Juglans Regia L.	Brissmoret and Combes	Compt.rend.141,838 (1905)
1906.	Carpinus Betulus L.	Alpers	Arch.Pharm.244,575 (1906)
1907.	Raspberry jam	Kunze-Krause	Pharmaceut.Praxis 6,336(1907)
1909.	Terminalia Chebula R. Punica Granatum L. Caesalpinia digyna Rottl Quercus aegilops L. Terminalia Catappa (L) Lyons Quercus Robur L.	Nierenstein	Chem.Zeitg.33,87 (1909)
1921.	Raspberry syrup	Kunze-Krause	Arch.Pharm.259,193 (1921)
1922.	Acer ginuala __.	Perkin	J.Chem.Soc.121,66 (1922)

A few other plants from which ellagic acid has been isolated are listed in Wehmer (Die Pflanzenstoffe, 1931).

Physical Properties.

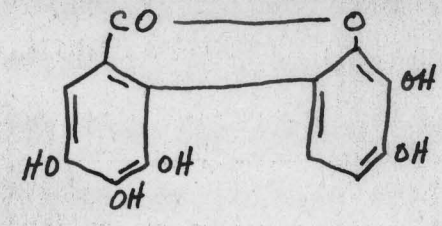
Ellagic acid is a pale yellow, microcrystalline powder, forming rhombic prisms or prismatic needles. It is practically insoluble in all the common solvents. Boiling water, ethyl and methyl alcohols have a slight solvent effect. It is soluble in boiling pyridine, from which it may be crystallized to form buff colored crystals; these crystals contain pyridine of crystallisation, which may be removed by washing with alcohol, yielding

the pale yellow acid (6).

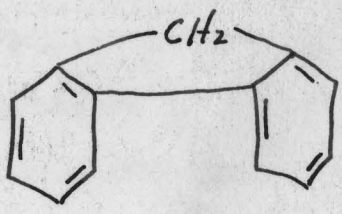
Air dried ellagic acid contains 2 molecules of water of crystallisation which are lost upon drying to constant weight at 100°. Alpers (10) is of the opinion that when it is dried at higher timperatures it loses constitutional water. Ellagic acid does not melt below 360°: When heated to higher temperatures it sublimes with the production of glistening yellow crystals, the identification of which has not been recorded in the literature.

Chemical Properties.

Ellagic acid is very resistant to the usual chemical reagents which might be expected to react with a compound of its structure. It can be acetylated and benzolyated only with great difficulty. The halogen acids have no effect upon its structure or physical appearance (11). Fusion with caustic potash yields pentahydroxy-diphenylmethylolid (6):



Upon distillation with zinc dust it yields the hydrocarbon, fluorene (5):



It dissolves in dilute alkalies with a deep yellow color which upon exposure to air becomes reddish-yellow, with the separation of dark colored crystals of the alkali glaucomelanate, $K_2C_{12}H_4O_7$. (7). Upon acidification of the dilute alkali solution the acid is obtained again.

Ellagic acid is colored green, changing to a dark blue, by ferric chloride solution. With nitric acid containing a small amount of nitrous acid and with dilution it gives a blood red coloration (Griessmayer's reaction), which is considered to be characteristic (8), although it has been considered by some to be due to an impurity (9).

Derivatives.

The following "salts" of ellagic acid have been reported:

$Na_2C_{14}H_4O_8$ - by heating ellagic acid with NaOH solution and precipitating with CO_2; a bright yellow crystalline powder (7).

$NaC_{14}H_5O_8$ - warming gallic acid ethyl ester with soda solution; citron yellow silky crystals (12).

$K_2C_{14}H_4O_8.KOH$ - Citron yellow, microscopic prisms (7).

$K_2C_{14}H_4O_8$ - microcrystalline (7).

$Ba_3(C_{14}H_5O_9)_2$ (at 140°) - yellow powder (7).

$Pb.C_{14}H_4O_8.H_2O$ - yellow, amorphous (7).

$KC_{14}H_5O_8$ - minute yellow needles (13).

The following derivatives of ellagic acid have been prepared and reported in the literature:

Tetraacetyl ellagic acid (16), (17), (6), (11), prepared

by the action of boiling acetic anhydride on ellagic acid; glistening white crystals, M.P. 343-346°.

Tetrabenzoyl ellagic acid (18); colorless needles, M.P. 332-333°.

Tetragalloyl ellagic acid (19), prepared by the action of tricarbonethoxy galloyl chloride on ellagic acid in alkaline solution; small yellow needles, M.P. 297-300°, with decomposition, gives gelatin reaction for a tannin.

Leucoellagic acid (20), $C_{14}H_{10}O_8$, by reduction of ellagic acid; colorless needles, M.P. 294-296°, with decomposition,

Ellagic acid monomethyl ether (21), by the action of methyl iodide and KOH on ellagic acid; pale yellow crystalline powder, which upon heating with acetic anhydride forms the diacetyl ellagic acid monomethyl ether, $C_{14}H_3O_7(OCH_3)(C_2H_3O)_2$, a crystalline powder.

Ellagic acid dimethyl ether (21), by longer action of methyl iodide and KOH on ellagic acid; a crystalline powder.

Ellagic acid tetramethyl ether (21) (22), by the action of diazomethane on ellagic acid in ether suspension; a colorless microcrystalline powder.

The following reduction products of ellagic acid have also been reported:

Hexaoxybiphenyl (20), obtained by electrolytic reduction, M.P. 312-317°.

Pentaoxybiphenyl methylolid (23), by the action of sodium amalgam.

Hexaoxybiphenyl (24), by the action of sodium amalgam

and by the action of KOH (11).

Formation and Preparation.

Synthetic ellagic acid, so called, has been prepared by a number of ways, among which the following are noteworthy:

Oxidation of gallic acid by arsenic acid (14).

Treatment of ethyl gallate with Na_2CO_3 in air (12).

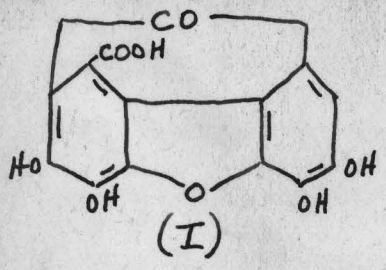
Oxidation of gallic acid with iodine (8).

Oxidation of gallic acid in acetic acid solution with potassium persulphate in the presence of H_2SO_4 (6).

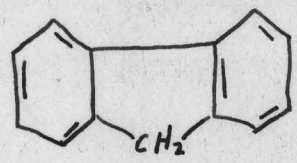
Treatment of alcoholic solution of gallotannin with KOH in the presence of air (15).

Structure.

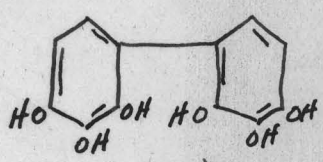
Barth and Goldschmidt (11) in 1879 suggested a structural formula for ellagic acid which was further developed by Goldschmidt and Jahoda (18) into (I). The evidence for this formula was that ellagic acid on distillation with zinc dust yields fluorene, to which they wrongly assigned formula II, and that on fusion with KOH it was supposed, wrongly, to yield hexahydroxydiphenyl (III).



(I)



(II)

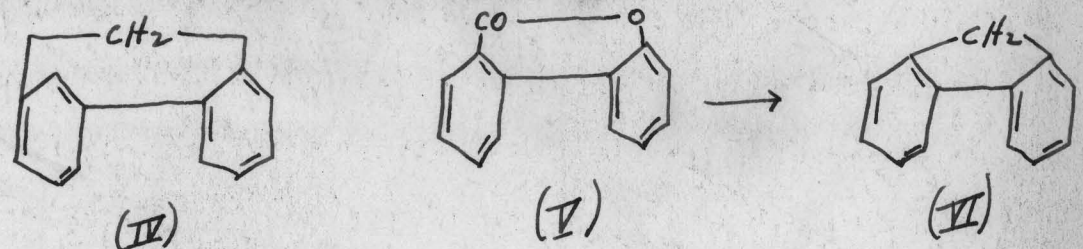


(III)

Schiff (4) in 1897 had suggested the correct formula for ellagic acid to explain its production from digallic acid, and also from his observation that the compound formed a tetra-

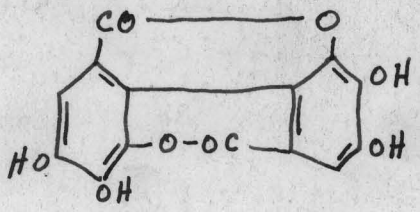
acetyl derivative. His work, however, was overshadowed by that of Goldschmidt.

Graebe (5), however, in 1903 attacked Goldschmidt's work by showing that a compound of his structure would yield on distillation with zinc dust, not fluorene, but an isomeric hydrocarbon (IV). Graebe and Schestakow (25) in 1895 had shown that all substances of type (V) (diphenylmethylolid) would yield fluorene (VI) on zinc dust distillation:

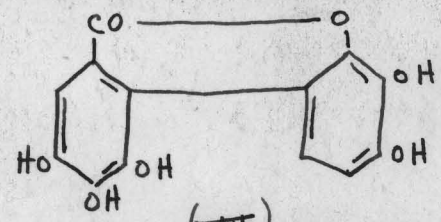


(IV) (V) (VI)

Graebe's work thus revived Schiff's original formula for ellagic acid as the correct one:



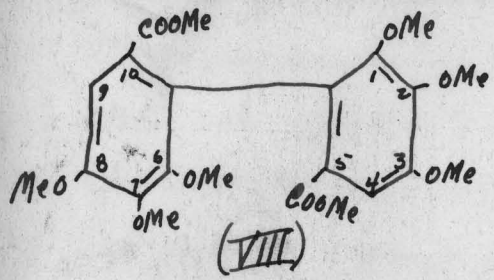
Perkin and Nierenstein (6) in 1905 further confirmed Schiff's formula when they studied synthetic ellagic acid, produced by the oxidation of gallic acid in acid solution, and showed that on treatment with alkali ellagic acid yields pentahydroxy diphenylmethylolid (VII) and not the supposed hexahydroxybiphenyl of Goldschmidt:



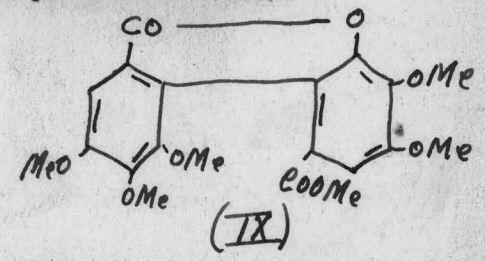
(VII)

They also obtained fluorene on zinc dust distillation.

Herzig and Pollak (22) in 1908 obtained further corroboration of Schiff's formula by a study of the methylation products of ellagic acid. They prepared the tetramethyl ether of ellagic acid by prolonged action of diazomethane on an ether suspension. By treatment of this tetramethyl ether with KOH and methyl iodide they obtained an ether-ester of the acid, M.P. 109-111°, which was diphenyl-1,2,3,6,7,8,-hexamethoxy-5,10-dicarbonic acid methyl ester (VIII), together with a small amount of the intermediary product, diphenyl methylolid-2,3,6,7,8-pentamethoxy-5-carbonic acid methyl ester (IX):

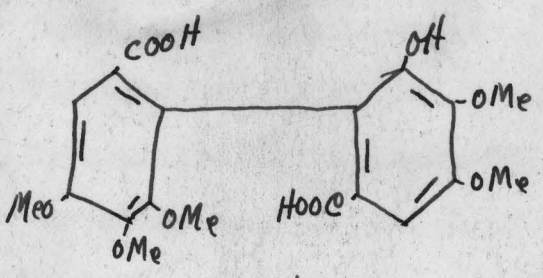


(VIII)

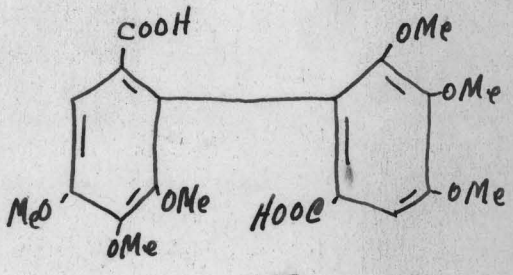


(IX)

Saponification of this intermediary lactone ether-ester (IX) with alcoholic KOH yielded diphenyl-2,3,6,7,8-pentamethoxy-1-hydroxy-5,10-dicarbonic acid (X), M.P. 200-203°, while saponification of the end product of the methylation (VIII) above yielded diphenyl-1,2,3,6,7,8-hexamethoxy-5,10-dicarbonic acid (XI), M.P. 238-240°:



(X)



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