PART I

AN INVESTIGATION INTO THE DRUG CONTENT OF CERTAIN MEDICINAL PLANTS

PART II

THE QUANTITATIVE DETERMINATION OF ETHER IN ETHER, ALCOHOL AND WATER MIXTURES

by

ALICE GRETA WINTER

A Thesis submitted for the Degree of

MASTER OF ARTS

in the Department of

CHEMISTRY

UNIVERSITY OF BRITISH COLUMBIA

April 1927

TABLE OF CONTENTS

PART I

AN INVESTIGATION OF THE DRUG CONTENT OF CERTAIN MEDICINAL PLANTS.

TABLE OF CONTENTS.(Cont'd)

PART I

AN INVESTIGATION INTO THE DRUG CONTENT OF CERTAIN MEDICINAL PLANTS.

GENERAL INTRODUCTION

The present work consists of a report on the analysis of certain drug plants grown at the Botanical Gardens of the University of British Columbia. The plants investigated were Hydrastis canadensis, Atropa belladonna, Podophyllum peltatum, and Populus Trichocarpa. These plants are all important commercially, as they are of value in medicine. Besides the results of the analyses, an effort has been made in each case to set forth the possibilities of growing the herbs under cultivation on a commercial basis.

HYDRASTIS CANADENSIS

INTRODUCTION

For the past twenty years a wide interest has been shown in the cultivation of Hydrastis canadensis, or Golden Seal, as it is more commonly called. Two different facts are responsible for this, a growth in the demand, due to an increasing knowledge of the usefulness of this drug in medicine, and a gradual depletion of the natural sources of supply. These two causes, as might be expected, have greatly increased the market price, which has risen from about twenty cents a pound in 1894 to from ten to sixteen dollars a pound at the present time. Such a marked change in price naturally turned the attention of many drug-collectors and others to the possibility of cultivating this herb. At the present

time it is found that Hydrastis can be very successfully grown under special conditions, and that it is possible to increase the alkaloidal content of the herb over that which it possesses in the wild state, thus rendering it more valuable.

HISTORY AND USES

The earliest settlers of America learned of the virtue of Golden Seal from the Indians, who used the roots as a medicine, and the yellow juice as a stain for their hands and faces. An infusion of the root in water was regarded as a specific for inflamed eyes and sore mouth, while the ground root was used as a tonic. Many different names, most of them derived from its yellow color, were applied, such as Indian dye, Indian tumeric, jaundice root, yellow puccoon, wild curcuma etc. Later it became generally known as Golden Seal, the name being derived from its yellow color and the peculiar seal-like scars left on the root by former annual stems. Around the year 1747 a demand for this herb was created by the eclectic school of practitioners, which led to it becoming an article of commerce. In 1860 it was made official by the Pharmacopoeia of the United States, and since then the demand has steadily increased. At present it is used as a remedy for certain digestive disorders, for catarrhal affections of the mucous membranes, in the latter instance being administered both internally and locally, and as a bitter tonic analogous to calumba.

HABITAT AND RANGE

In the earlier years of its use, until about 1900, enough Golden Seal occurred naturally to supply the demand. The plant is indigenous to North America, and is native from Minnesota to New York and Ontario, also south to Virginia and Missouri. Ohio, Indiana,

Kentucky and Virginia have, perhaps, been the largest producers. It is found growing in patches, chiefly on hillsides affording natural drainage, and in woods which supply both shade and an abundance of leaf mold. As the roots of this herb are the parts required, many of the natural sources of supply have been gradually destroyed by the drug-collectors, who showed no discretion in their choice of season, often leaving the plant no means of propagation. Then also the increase in the area of cultivated land with advancing civilization is destroying the forests, so that year by year the extent of wild growth decreases.

DESCRIPTION OF PLANT AND ROOTSTOCK

Golden Seal is a perennial, the plant reaching maturity, as far as harvesting for medicinal purposes is concerned, in four or five years. It has an erect, hairy stem and two leaves, palmately lobed, which are from ten to twelve inches in diameter. The rhizome and rootlets, which are the parts used in medicinal preparations, are bright yellow thoughout when fresh, and contain a quantity of yellow juice, which gives off a rank, nauseating odor. The rhizome is from one-third to one-quarter of an inch thick, and from one to two feet long, with fibrous yellow roots produced from the sides. When dry the rhizome appears knotty and wrinkled; of a dull brown color outside, and pale yellow inside. It has a characteristic, quinine-life odor, and bitter taste. Each plant produces one white flower. The seeds, which are green at first, turn to scarlet in July, when they should be harvested, and dark brown in the early part of August, if left on the plant.

GROWING CONDITIONS

It is a recognized fact that Hydrastis cannot be grown successfully in an open field, and that it is necessary to imitate natural conditions as much as possible. Two different methods of accomplishing this result present themselves; the first to grow the herb in a plantation of trees, thus obtaining conditions identical with those under which it is found in nature, and the other to provide artificial shade. Both these methods have their disadvantages, the trees in the former depriving the plant of much of the nourishment and moisture in the soil, while the latter method is naturally the most expensive.

The plants at the Botanical Gardens were grown under artificial shade. Ordinary laths, about two inches wide, were used to make the shelter, supported by posts driven into the ground at intervals of eight feet. The shed was about eight feet in height, completely covered with laths. In one-half the frame the laths were their own width apart, thus providing fifty per cent shade; in the other half they were placed closer together, in order to afford seventy-five per cent shade. The latter is considered to best represent the conditions in a forest of average density, and the plants were found to need this amount of protection.

In order to secure good drainage, beds were made raised about four inches above the level of the ground, the edges being supported by boards. A light rather sandy soil was used, into which a quantity of leaf mold had been introduced in order to secure the fertility and moisture retaining properties of forest soil. The plants were grown from seed, and allowed five years to reach maturity. Each fall they were covered up with leaf mold to protect the roots in the winter. The plant

will also propagete itself by the formation of new plants on the long slim fibre roots. After the first year, yellow buds will appear on the rhizome, which, if not disturbed, will send up new stalks. If the rhizomes are taken up and divided, a bud being left on each piece, and planted at a distance from each other, separate plants will appear, which will reach maturity in four years.

The bed in which the Hydrastis was grown measured 216 sq. ft. The plants were placed about I ft. apart. From this area, 60 lb., green weight, was obtained. This would indicate that an acre grown under like conditions should yield about 10,000 lb, green weight, which is equivalent to 3,500 lb. dry weight. This compares favorably with yields obtained in other localities.

HARVESTING AND DRYING

As the drug content of the roots varies considerably with the season, the crop should be lifted in either September or October when the leaves are dying down. Spring dug roots do not contain as much alkaloid, and so command a lower price, than fall dug roots. The roots sent in for analysis were lifted on September 25, 1925. All the loose soil was shaken out, they were well washed, the moisture allowed to drain off, and then weighed (green weight). They were then spread in thin layers on wooden desks covered with paper, which served to catch any fibrous roots becoming detached from the rootstock. The drying was facilitated by turning the roots over each day. This was necessary on account of the tendency of the roots to mold if the drying is too slow. The roots were reweighed at intervals, until after two weeks no further loss of weight was noticed, and they were assumed to be completely airdried. It was found that the roots lost approximately 65% of their weight in drying.

DISCUSSION OF ANALYSIS

Hydrastis canadensis is valued on account of the alkaloids which the roots contain. Of these the two most important are Hydrastine and Berberine. The roots also contain small quantities of canadine, meconin, and phytostearin. It is possible to separate the berberine and hydrastine owing to their different solubilities in ether, and to determine each separately.

After having been thoroughly air-dried, the root was ground up in an iron mortar until the powder would pass through an eighty mesh seive. It was then well mixed, and a sample taken by the method of quartering. This sample was then desiccated to constant weight, when it was ready for analysis. The ground root was kept in a glass container, tightly stoppered, and protected from the light.

Two different methods of analysis were used, that of the United States Pharmacopoeia, Ninth Revision, which is the official method for obtaining the total alkaloids, and a method of Gordin and Prescott[I] for the separation and determination of the hydrastine and berberine.

U. S. P. METHOD

Introduce 10 g. of the powdered root into a 250 c.c. flask, and add 100 c.c. ether. Stopper, shake well, and allow to stand for 10 min., then add 5 c.c. of NH_4OH water, and shake the flask vigorously every 10 min. for 2 hrs. Now add 15 c.c. distilled water, again shake the flask well, and when the drug has settled decant 100 c.c. soln., representing 5 grams of the root. Filter the soln. through a pledget of

[I] Arch. Pharm., 1899, 237, 441.

purified cotton into a separator, and rinse the graduate and cotton with a little ether. Completely extract the alkaloids from the soln, by shaking out repeatedly with weak H_2SO_4. Collect acid washings in a separator, add NH_4OH water until decidedly alkaline, and completely extract alkaloids by shaking out repeatedly with ether. Evaporate combined ether washings to dryness, dry the residue to constant weight at 100°, and weigh. Weight is amt. of ether soluble alkaloids from 5 g. Hydrastis.

METHOD OF GORDIN AND PRESCOTT

Stir 10 g. of the powdered root into a paste with a few c.c. of a mixture of alcohol, concentrated NH_4OH, and ether, in the proportions 1:1:6, and kept in a stoppered jar for several hours, (overnight). Dry the mixture in a current of air, until all the NH_4OH has volatilized, and afterwards in a vacuum over H_2SO_4 for 5 - 6 hrs. Transfer the dry substance to a Soxhlet extractor, the jar being rinsed with powdered $Ba(NO_3)_2$, and extracted with 40 - 50 c.c. absolute ether until the residue from the evaporation of a few drops of the extract gives no reaction with Mayer's or Wagner's reagents. The ethereal extract contains the hydratsine. Pass a current of air through the Soxhlet tube until all the ether has evaporated, and extract the residue with 40 - 50 c.c. of alcohol. Continue extraction until the extract is colorless. Wash the alcohol extract into an evaporating dish together with a little hot water and dilute HAc, and evaporate with the addition of water until all the alcohol is expelled. Add a further small quantity of dilute HAc, cool liquid, filter into a 300 - 400 c.c. Erlenmeyer flask and wash the filter. Treat filtrate with 6 - 8 c.c. of acetone, and a 10% soln. of NaOH drop by drop until the precipitate formed no longer disappears upon shaking, and the liquid acquires a strongly alkaline reaction. Stopper flask, shake

in a circular direction for 10 - 15 min., and then allow to stand for 2 - 3 hrs. Wash crystals of berberine-acetone formed until the washings are colorless, return to flask, and treat with 4 - 5 c.c. of 5% H_2SO_4 and water up to 100 - 200 c.c. Heat flask in hot water until ppt. dissolves, transfer to long-necked flask, and boil gently for 2 hrs., adding water when necessary. Cool liquid, mix with 100 c.c. of N/20 KI soln., dilute mixture to 1 litre, and allow to stand overnight. Filter liquid, mix 500 c.c. of the filtrate with 50 c.c. of N/20 $AgNO_3$ soln., add a little HNO_3, and dilute to 1 litre. Shake, filter, and titrate 500 c.c. of the filtrate with N/40 NH_4CNS, using ferric alum as indicator. Twice the number of c.c. of NH_4CNS used is equal to the number of c.c. of N/20 KI soln. consumed by the berberine in 10 g. of root. The number of c.c. of N/20 KI soln. consumed multiplied by 0.167125 gives the percentage of anhydrous berberine.

RESULTS OF ANALYSES

The U.S.P. standard for the "ether soluble" alkaloids in Hydrastis is not less than 2.5%. Owing to the fact that water is present, much of the berberine as well as the hydrastine must also be present in the final residue. Great difficulty was encountered in the procedure owing to the presence of oil and resinous matter which was extracted with the alkaloid. This difficulty was finally overcome by first treating the powdered root with petroleum ether, in which both hydrastine and berberine are insoluble. This removed the fixed oil. By this method of analysis the drug showed an alkaloidal content of 2.8%, which is above the Pharmacopoeia standard.

By Gordin and Prescott's method, the drug was found to contain 1.5% hydrastine and 1.7% berberine, which gives a total

alkaloidal content of 3.2%. The difference in the two results is doubtlessly due to the fact that all the berberine is not extracted in the U. S. P. process.

These results compare favorably with other reports on the alkaloidal content of various crops. Harding, [2] who has grown hydrastis for many years, gives the average amount of alkaloid as 2.6 to 2.7%. Perrin,[3] who analysed both the wild and cultivated root, gives 2.5% as the average amount in the former, and 3.1 % in the latter.

CONCLUSION

The price paid at the present time for Hydrastis offers a great inducement to anyone contemplating growing it on a commercial scale. The highest current price for fall dug roots is $16.75.lb., this being for the dried root. The price of this herb fluctuated a good deal some years ago, owing to the alternate oversupply and scarcity, but this was when a good supply was at hand in the forests. But a fairly steady advance in price over the last twenty years can be noted. In 1904 the demand for the root was increasing, and the price rose to $1.40 a lb., the first time that a noticeably high price was paid. In 1911 the price rose to $4.50 a lb. The price increased yearly until 1920, when it reached $12.00 a lb. In the last six years the price has risen from $12.00 to $16.00, which gives a fair idea of the stability of the market.

Calculating on the basis of 3,500 lb. to the acre, the gross returns from an acre of Hydrastis should be somewhere in the neighborhood of $50, 000.

[2] Ginseng and Other Medicinal Plants.

[3] Amer. J. Pharm., 1904, 153, 226

ATROPA BELLADONNA

INTRODUCTION

Atropa belladonna, or deadly nightshade, has been known from early times on account of its poisonous properties, and its mydriatic action upon the eyes. The name, (from the Italian "beautiful lady"), is said to be derived from the fact that the berries were used as a cosmetic. Preparations from the leaves, roots, and stems have a wide application in pharmacology both for their mydriatic property, and their paralytic effect on the muscles and the central nervous system.

DESCRIPTION OF PLANT

Belladonna is a perennial belonging to the natural order Solanaceae. It is a native of the region from southern Europe to India, but is widely naturalized in most civilized countries. It is a beautiful, spreading plant, sometimes attaining the height of six feet. The leaves are entire and ovate, and dark green in color. Purple flowers occur either singly or in pairs, and later black, shiny berries, about the size of a currant, appear. The whole plant has a disagreeable, bitter odor. The rootstock is long, thick and fleshy, with small rootlets at the sides and end.

GROWING CONDITIONS

One plant of Atropa belladonna was grown at the Botanical Gardens. This was grown from seed, which was planted in light, rather sandy soil, on June 20, I923. The herb was grown in an open, sunny place, on land which had a gentle slope to ensure good drainage. At the time of lifting, October 6, I925, the plant was five feet tall,

covering an area of about 4 sq. ft. The yield obtained from this specimen was I lb. of leaves and I0 lb. of root, green weight. In order for the plants to attain full growth it would be necessary to allow 2 sq. yd. to a plant. This would give a yield of 2400 lb. of leaves and 24,000 lb. of root to the acre, or I200 lb. of leaves, and I0,800 lb. of root dry wt. Upon drying (under the same conditions as the Hydrastis), the leaves lose 50%, the roots 55%, of their moisture.

DISCUSSION OF ANALYSIS

The chief alkaloids of Atropa belladonna are hyoscyamine and atropine, although a small quantity of hyoscine is also present. No reliable methods have been worked out for the separation of these two alkaloids, as the hyoscyamine is readily converted into atropine in the process of extraction. Therefore the only determination made is that of total alkaloid.

The leaves and root were each ground in an iron mortar until they would pass through an eighty mesh sieve; desiccated to constant weight, and were then ready for analysis. The roots and leaves were kept in a closed container away from the light.

The method of the United States Pharmacopoeia was used for the analysis of both leaves and roots. Then other samples were treated using a slight modification of this procedure, in that a Soxhlet apparatus was used for the extraction, and the final residue was first weighed as a check before titrating.

U. S. P. METHOD

Roots. Introduce I5 g. of powdered root into a 250 c.c. flask, and add I50 c.c. of a mixture of $CHCl_3$, I vol., and Et_2O, 2 vol. Stopper, shake well, and allow to stand for I0 min., then add 5 c.c. of

NH_4OH water and shake the flask vigorously every I0 min. for two hrs. Now add I5 c.c. of distilled water, again shake the flask well, and when the drug has settled decant I00 c.c. of the soln. representing I0 g. root. Filter soln. through a pledget of purified cotton into a separator, and completely extract the alkaloids from the soln. by shaking out repeatedly with weak H_2SO_4. Collect acid washings in a separator, add NH_4OH water until decidedly alkaline, and completely extract the alkaloids by shaking out repeatedly with $CHCl_3$. Evaporate combined $CHCl_3$ washings to dryness, dissolve the alkaloids from the residue in exactly 5 c.c. of N/I0 H_2SO_4, and titrate the excess of acid with N/50 KOH, using cochineal as indicator. Every c.c. of N/I0 H_2SO_4 consumed corresponds to 28.92 milligrams of the total alkaloids.

Leaves. Proceed as above, using I5 g. of leaves, and increasing the amount of water addad after maceration to 25 c.c., and, before titration, treating the final residue twice with 5 c.c. of Et_2O, evaporating to dryness each time.

RESULTS AND CONCLUSION

By the U.S.P. method, the amounts of total alkaloids found were: leaves, .55%, roots .64%. By using the Soxhlet extractor, more of the alkaloid was removed, giving the result of leaves, .60%, roots .67%. The U.S.P. standard for alkaloidal content is leaves, not less than .3%, and roots not less than .45%. Other reports have shown that there is a great variation in the amounts of alkaloid present, some investigators reporting more in the cultivated plant, others claiming to have found more in the wild. It is a recognized fact that second year plants contain more alkaloid than those only one year old.

PODOPHYLLUM PELTATUM.

INTRODUCTION.

Podophyllum peltatum, or May apple, is another useful drug plant. Other common names by which it is known in various localities are mandrake, wild lemon, hog apple, devil's apple, and vegetable calomel. Although not of such importance as Golden Seal, it has a fairly wide application as a catharic. Of later years the use has diminished somewhat due to certain objectionable properties which it possesses, such as the poisonous effect of large doses.

DESCRIPTION OF PLANT.

The plant is an indigenous perennial, belonging to the barberry family (Berberidacae). It is found from western Quebec to Minnesota, and south to Florida and Texas, growing in low woods, usually in dense patches. It is of erect growth, from one-half to two feet in height, with smooth, dark green foliage, and is a conspicuous plant throughout its range in early spring. The leaves are two in number, and about one foot in diameter. A waxy white, solitary flower appears in May, and later a lemon-shaped fruit, which is first green, and turns yellow upon ripening. This fruit is edible, and has a sweet, honey-like flavor.

The root is the important part of the plant for medicinal purposes. The horizontally creeping rootstock is from one to five feet in length. It is flexible, smooth and round; dark brown on the outside and whitish or fleshy within. At intervals of a few inches are thickened joints, with tufts of roots on the lower side.

GROWING CONDITIONS

The Podophyllum was grown under identical conditions to the Golden Seal. It was grown from seed, and was five years old at the time of lifting, Oct. 20, 1925. From an area of 80 sq. ft., a yield of 16 lb. of root (green weight) was produced, which would indicate a yield of approximately 8000 lb. to the acre, or about 3200 lb. dry weight. The root lost about 60% of its weight upon drying, which was done in the same way as the Hydrastis.

DISCUSSION OF ANALYSIS

The Podophyllum root contains a resin which is used in medicinal preparations. The amount of resin present was determined by the United States Pharmacopoeia method. The root was ground up into a fine powder(until it would pass through an eighty mesh sieve), and then desiccated to constant weight.

U.S.P. METHOD

Moisten 10 g. of powder with 500 c.c. of EtOH, and pack in a cylindrical percolator; then add enough EtOH to saturate the powder and leave a stratum above it. When liquid begins to drop from the percolator, close lower orifice, closely cover percolator, and macerate for 48 hrs. Then allow percolation to proceed, gradually adding EtOH until percolate ceases to produce more than a slight turbidity when introduced into water. Distil off the EtOH until the percolate is reduced to the consistency of thin syrup, then pour slowly, with constant stirring into 1 litre of water, mixed with 10 c.c. of HCl and cooled below 10° C. When ppt. has settled decant liquid, and wash ppt. twice with water by decantation. Spread resin in a thin layer upon a strainer, and dry by

exposure to the air., in a cool place, protected from the light.

RESULTS

The root investigated was found to contain 3.I % of resin. The Pharmacopoeia standard for Podophyllum is given as not less than 3.0 % resin.

POPULUS TRICHOCARPA

INTRODUCTION

Balm of Gilead, or Mecca balsam, an exudation from the Balsamodendron gileadense, has been of common use in pharmacology for some years as a stimulant to the mucous membranes in chronic bronchitis and catarrh. As a substitute for this the balsam from the buds of the Populus trichocarpa is used. This balsam is considered if anything superior to the original Balm of Gilead, by which name it is sometimes erroneously called.

GROWING CONDITIONS

The Populus trichocarpa is a tall, branching tree, and consequently it is difficult to gather the buds. It was thought that it would be possible to grow cuttings which would not be allowed to attain a height which would make harvesting difficult. Accordingly cuttings were planted at intervals of about three feet. The buds, which contain the balsam, were collected in the latter part of January from one year old cuttings. As the crop was in nowway representitive of the acreage, owing to the youth of the trees, no calculations were as to the yields possible per acre.

DETERMINATION OF BALSAM IN BUDS

When the buds were recieved, they were weighed and placed in a Soxhlet extractor, and the balsam completely extracted with ether. The ether was then distilled off, leaving a thick brown viscous mass, of highly agreeable aromatic odor. The yield of balsam from the buds was 31 %. It was completely soluble in ether and alcohol, partly soluble in chloroform, and insoluble in water.

DETERMINATION OF SPECIFIC GRAVITY

The density of the balsam was obtained through the method of mixtures. A 10 c.c. specific gravity bottle was cleaned, dried and weighed; then filled with distilled water at 20° C., and again weighed. The bottle was then emptied, dried, and about 2 c.c. of the balsam introduced by means of a glass rod. It was then weighed. Distilled water was poured on the balsam until the bottle was completely filled, and the bottle and contents weighed at 20° C. From this determination the density of the balsan was found to be 1.134 at 20° C.

SAPONIFICATION VALUE

The saponification value was found as follows: 1 g. of balsam was dissolved in 10 c.c. of alcohol and treated with 25 c. c. of N/2 alcoholic KOH. The soln was then boiled on a steam bath under a reflux for one hour, when it was diluted with alcohol, and titrated with N/10 H_2SO_4. Using this method the average of several closely agreeing values was 170.8.

ACID AND ESTER VALUES

The acid value was found by direct titration., 2 c. c. of the balsam being dissolved in alcohol, and titrated with N/10 H_2SO_4

The acid value was found to be 88.0.

The ester value is given by the differ ence between the saponification and acid values, and is accordingly 82.0.

CONCLUSION

The different constants of this balsam differ greatly from those of the Mecca balsam. The substance also contains a quantity of volatile oil, which has not yet been investigated.

DETERMINATION OF ETHER IN ETHER, ALCOHOL, AND WATER MIXTURES.

The purpose of this investigation was to find a practical method of analysis for the ether, alcohol and water mixtures resulting from the catalytic dehydration of alcohol to ether by means of Al_2O_3. In many of these mixtures the yield of ether is sufficiently high to cause a separation into two layers. It was necessary to take this fact into consideration in the choice of a method of analysis.

A chemical method, that of oxidation by means of $K\,Mn\,O_4$ in an acid solution, was first tried. It was thought that it would be possible to oxidize all the alcohol to acetic acid without an appreciable amount of the ether being touched, and thereby changing the conditions in a second sample, to oxidize both the ether and the alcohol. The ether could then be obtained by difference. This was found to be unsatisfactory in practice, owing to the varied amount of ether attacked in the alcohol oxidation, and the tendency of the $Mn\,O_2$ to precipitate out. A similiar method, using $K_2Cr_2O_7$ as oxidizing agent, had already been tried out in this laboratory with unsatisfactory results.

The following method of salting out the ether from the mixture with saturated Na Cl solution was then tried, giving results accurate to within 1%. Wolff, Chem. Ztg. 34, 1193, uses the principle of salting out for the determination of ether and benzene in alcohol. An adaptation of Wolff's method has also been used by R. N. Pease and Chi Chao Yung, J. Amer. Chem. Soc., Vol. 46, 1924, 2397, for their analysis in the production of ether from alcohol.

The latter authors found that upon shaking a mixture of

ether and alcohol with about four times its volume of salt solution, the greater part of the ether separated out. They show a curve giving the correction, the true volume of the ether being plotted against the volume separating. This correction is necessary owing to the solubility of the ether in the alcohol and water of the lower layer , and also the solubility of the alcohol in the ether of the upper layer. The solubility of the water in the ether is negligible. A shrinkage in volume also takes place owing to the fact that the initial volume of ether is measured at room temperature, and that of the upper layer at 10^{0} C. The mixtures of ether, alcohol and water used were those which would be produced in the different percentage conversions of 50 g. of alcohol to ether. Their curve shows that when the yield of ether is low, the separation is greater than the true volume, and that when the yield is high, the separation is less than the true volume. Preliminary trials were made with corresponding mixtures, in order to see whether these values for the correction could be duplicated. The values obtained coincided with those given for the higher yields, but not for the lower, the volume separating being always less than the true volume. Accordingly it was thought necessary to carry out a series of determinations, in order that this method could be used for the mixtures under consideration.

It was found convenient to work with a smaller volume, 20 c.c. being chosen as the most suitable. For this purpose a 50 c.c. burette, sealed to the neck of a 100 c.c. volumetric flask, was used. Amounts of ether and alcohol in the required proportions were run into the flask from two burettes, the room temperature being noted. The flask was then corked, and suspended in a water bath at 10^{0} C. After the addition of 100c.c. of saturated salt solution, the flask was tightly corked, and

vigorously shaken for a few minutes. This vigorous shaking was continued until complete equilibrium between the two layers had been reached. The volume of the upper layer was then read at 10^{0} C.

The first mixtures used were of ether and alcohol alone, having a total volume of 20 c.c., and varying in composition from 100% ether to 100% alcohol.

TABLE I

Measured Vol. Ether	Measured Vol. Alcohol	Separation	Correction
20.0 c.c.	0 c.c.	18.6 c.c.	1.4
18.1 c.c.	1.9 c.c.	16.8 c.c.	1.3
14.8 c.c.	5.2 c.c.	13.6 c.c.	1.2
12.0 c.c.	8.0 c.c.	10.9 c.c.	1.1
9.9 c.c.	10.1 c.c.	8.9 c.c.	1.0
7.6 c.c.	12.4 c.c.	6.7 c.c.	0.9
5.8 c.c.	14.2 c.c.	4.9 c.c.	0.9
4.2 c.c.	15.8 c.c.	2.9 c.c.	1.3
2.9 c.c.	17.1 c.c.	0.7 c.c.	2.2

Beyond this point no appreciable separation occurred, the small volume of ether all dissolving in the bottom layer. It will be seen from this table that when the alcohol is more than three times the volume of the ether, the results become inaccurate.

To show the effect of small quantities of water on the separation, this determination was repeated, using the same amounts of ether and alcohol, but adding water in amounts varying from 1 to 5 c.c. An excess of solid salt was present. The addition of water had no apparent effect on the separation, the values obtained coinciding with

those of Table I .Therefore if 20 c.c. of an ether, alcohol and water mixture are taken for analysis, the only influence of the water would be to make the volume of ether and alcohol smaller in comparision to the amount of salt solution. In the case cited above, the water was added in addition to the 20 c.c. of ether and alcohol, and so the proportion of salt solution to ether and alcohol remained unchanged.

To determine what difference the amount of salt solution present makes in the separation, the volume of the mixture was varied, that of the salt solution being kept constant. With pure ether, as might be expected, a constant amount dissolves in the salt solution, independent of the total amount of ether present.This is due to the slight solubility of water in ether. When alcohol is present, however, the correction varies with the total volume of the mixture, the correction becoming greater as the volume decreases.This is in accordance with the partition law. The concentration of the alcohol in the water layer is diminished,and therefore it must also become less in the ether layer, causing a shrinkage in volume. Two different mixtures were tried, the proportion of ether to alcohol being kept constant while the total volume of the mixture varied from 20 to 5 c.c.; 100 c.c. of salt solution being used in each case.

TABLE II

Total Vol. Mixture	Measured Vol. Ether	Measured Vol. Alcohol	Separation	Correction
20 c.c.	14.8 c.c.	5.2 c.c.	13.6 c.c.	I.2
15 c.c.	11.1 c.c.	3.9 .c.c	9.85 c.c.	I. 25
10 c.c.	7.4 c.c.	2.6 c.c.	6.1 c.c.	I.3
5 c.c.	3.7 c.c.	I.3 c.c.	2.35 c.c.	I.35

TABLE III

Total Vol. Mixture	Measured Vol. Ether	Measured Vol. Alcohol	Separation	Correction
20 c.c.	9.9 c.c.	10.1 c.c.	8.9 c.c.	1.0
15 c.c.	7.4 c.c.	7.6 c.c.	6.25 c.c.	1.15
10 c.c.	4.95 c.c.	5.05 c.c.	3.65 c.c.	1.3
5 c.c.	2.5 c.c.	2.5 c.c.	1.1 c.c.	1.4

From this it is seen that changes in the correction which would occur if 5 c.c. of water were present in 20 c.c. of the mixture are only .05 c.c. in the first case , and .15 c.c. in the second case, and the change in the ratio of alcohol to ether is considerable in the two tables. In the dehydration of alcohol, when the percentage conversion of alcohol to ether is small, the water produced will not reache the amount of 2 c.c. for an original volume of 20c.c. alcohol. In the higher percentage yields, the water equivalent to this amount of alcohol will not be over 3 c.c., and as is seen above, the change in correction lies within the experimental error.

In the case of some of the alcohol being dehydrated to form ethylene, an excess of water would be present. Under properly regulated conditions, however, the conversion tomethylene will never be more than 5%, for which the amount of ~~ethylene~~ water formed is under 1 c.c. It was found that the presence of ethylene dissolved in the mixture did not affect the separation in any way.

Mixtures of ether, alcohol and water were now made up identical with those formed in the different percentage conversions of alcohol to ether by weight. Using the equation

$$2\ EtOH \longrightarrow Et_2O + H_2O$$

and taking the density of ether as .719 and that of alcohol as .793 at 15° C., the volumes corresponding to the different percentage conversions of alcohol to ether can be calculated. As has been previously stated, 20 c.c. of alcohol was found to be a convenient initial volume for the catalytic process., and the calculations were made from this. The following table gives the observed separation for the mixtures of composition indicated. The column headed percentage conversion shows the percentage of the total alcohol equivalent of the mixture which is equal to the ether present on the basis of the above equation. In practice a little more than 20 c.c. of alcohol was run over, in order that the conversion to ethylene might be taken into account. Therefore in practice the percentage conversions to ether cannot be taken directly from the table. These values served merely as a basis for the calculations. Owing to the shrinkage in volume upon mixing, the volume of the mixture resulting from an initial volume of 20 c.c. of alcohol is very nearly 20 c.c.

TABLE IV

% Conversion	Measured Vol. Ether	Measured Vol. Alc.	Measured Vol. Water	Separation	Correction
100%	17.8 c.c.	0 c.c.	3.1 c.c.	16.4 c.c.	1.4
90%	16.3 c.c.	2.0 c.c.	2.8 c.c.	15.05 c.c.	1.25
80%	14.3 c.c.	4.0 c.c.	2.5 c.c.	13.1 c.c.	1.2
70%	12.8 c.c.	5.7 c.c.	2.2 c.c.	11.7 c.c.	1.1
60%	10.7 c.c.	8.0 c.c.	1.9 c.c.	9.6 c.c.	1.1
50%	8.9 c.c.	10.0 c.c.	1.6 c.c.	7.9 c.c.	1.0
40%	6.9 c.c.	12.0 c.c.	1.2 c.c.	6.0 c.c.	0.9
30%	5.15 c.c.	13.8 c.c.	0.9 c.c.	4.2 c.c.	0.9
20%	3.3 c.c.	15.8 c.c.	0.6 c.c.	1.2 c.c.	2.1

As before, the values became inaccurate in mixtures representing conversions below 30% owing to the large amount of alcohol present. The corrections show no appreciable change from those of Table I. These values are for ether and alcohol in a definite series of ratios. In order to find how the correction would vary with different ratios of alcohol to ether, a fixed amount of ether was taken with varying amounts of alcohol, giving the following results.

TABLE V.

Measured Vol. Ether	Measured Vol. Alcohol	Separation	Correction
I4.0 c.c.	0 c.c.	I2.6 c.c.	I.4
I4.0 c.c.	2.0 c.c.	I2.65 c.c.	I.35
I4.0 c.c.	4.0 c.c.	I2.75 c.c.	I.25
I4.0 c.c.	6.0 c.c.	I2.8 c.c.	I.2

TABLE VI

I0.0 c.c.	0 c.c.	8.6 c.c.	I.4
I0.0 c.c.	2.0 c.c.	8.7 c.c.	I.3
I0.0 c.c.	4.0 c.c.	8.8 c.c.	I.2
I0.0 c.c.	6.0 c.c.	8.9 c.c.	I.I
I0.0 c.c.	8.0 c.c.	9.0 c.c.	I.0
I0.0 c.c.	I0.0 c.c.	9.05 c.c.	.95

It would appear from these values that there is a relatively large increase in the correction as the amount of alcohol decreases. However, for a decrease of 6 c.c. in the alcohol volume from that amount which would bring the total volume of the ether and alcohol mixture up to 20 c.c., the error introduced by using the values for the corrections

given in Table I is under 2% in both cases. Therefore forslight changes in the ratio of ether to alcohol the change in correction is small.

For the analysis of mixtures resulting from the conversion of alcohol to ether, this method is, therefore, accurate to within 1%, which is the limit of accuracy of the burette readings. When the ether has been determined in this manner, approximate values for the alcohol and water could be obtained from specific gravity measurements. If it should be more convenient to use larger volumes, the volumes of the mixture, salt solution, and correction, can be increased in the same ratio. For mixtures in which the ratio of the ether to the alcohol and water is not known, this method serves as an approximate determination, provided that the amount of ether is not less than one-third, and the quantity of water not more than one-half, the total volume of the mixture. Under these conditions the error is within 4%.

BIBLIOGRAPHY

Gordin, R. and Prescott, T. Archives of Pharmacology, 1894
Volume 237, page 441

Harding, A. H. "Ginseng and Other Medicinal Plants".
Published St. Louis, Mo., 1906

Henry, T.A. "The Vegetable Alkaloids"
Allens Commercial Organic Analysis, Vol. VI

Pease, R.N. and Yung, C.C. Journal of the American Chemical
Society, Vol. 46, 1924, page 2397

Perrins, H. American Jounal of Pharmacology, 1899
Vol. 253, page 642.

Pharmacopoeia of the United States, Ninth Revision.