

A STUDY OF SEVERAL SPECIES OF THE GENUS *MONARDA*.*,¹BY B. V. CHRISTENSEN² AND R. S. JUSTICE.³

2—CHEMICAL EXAMINATIONS OF ALCOHOLIC EXTRACTIVE AND MISCELLANEOUS DETERMINATIONS.

PART I. THE ALCOHOLIC EXTRACTIVE OF *MONARDA MENTHÆFOLIA*.⁴

Material used in the following investigation was collected during the months of June and July 1931, while the plants were in flower in the southeastern part of Wyoming and the adjacent portion of Colorado in the counties of Albany and Larimer, respectively. The fresh plants were identified by Professor Aven Nelson of the Botany Department of the University of Wyoming.

It may be of some importance to record the fact that these plants were collected at altitudes ranging from 5000 to 7000 feet. The soil was gravelly and was high in iron oxide content as manifested by the reddish color characteristic of the Sherman Hill Gravel obtained from that locality.

The material was air dried in a cool room immediately after collection. A representative sample of the total amount collected was separated as quantitatively as possible into flower heads, leaves, stems and roots, and the percentage of each was computed with reference to the total weights of the several parts. Inasmuch as the roots retained by the plants cannot be considered representative, the percentages are also computed with reference to the overground portion of the plant as indicated in the following table:

TABLE I.—PERCENTAGES OF THE PARTS OF *MONARDA MENTHÆFOLIA*.

	Entire Plant.	Overground Plant.
Flower Heads	8.76%	9.21%
Leaves	40.76%	42.88%
Stems	45.55%	47.91%
Roots	4.93%	0.00%
Total	100.00%	100.00%

The laboratory work for the following investigation was carried out at the University of Wisconsin. Time permitted a study of the leaves and flower heads only.

Twenty-four and one-tenth Kg. of comminuted leaves and flower heads were extracted with 95 per cent alcohol in a Lloyd extractor. The removal of the excess alcohol in the resulting extract was carried out under reduced pressure. The resulting, semi-solid extract weighed 4040 Gm. or 16.77 per cent of the dried plant material.

In order to remove the volatile as well as the fatty oil, the alcoholic concentrate was repeatedly shaken with petroleum ether. From the combined petroleum-ether solutions the solvent was recovered for the most part by distillation. The residue weighed 1180 Gm.

The petroleum-ether extractive and the residue of the alcoholic extract were both steam distilled. The steam distillation and the petroleum-ether extraction naturally separated the alcohol extractive into the following fractions:

* Scientific Section, A. PH. A., Dallas meeting, 1936.

¹ From a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Florida, August 1935.

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⁴ See *JOUR. A. PH. A.*, 25, 850 (1936), for preliminary report.

(1) The non-volatile products soluble in petroleum ether consisting of the fatty oil. This weighed 872 Gm. corresponding to 3.62 per cent of the air-dried plant material.

(2) The volatile products soluble in petroleum ether consisting of the volatile oil. Two hundred and sixty-six cc. of the original oil were obtained and in addition 41 Gm. were obtained by cohobation. The total corresponds to 1.26 per cent of the plant material.

(3) The water-insoluble portion of the alcoholic extractive previously deprived of its petroleum-ether soluble material. This weighed 687 Gm. corresponding to 2.85 per cent of the plant material.

(4) The water-soluble portion of the alcoholic extract previously deprived of its petroleum-ether soluble material. This extract weighed 2174 Gm. corresponding to 9.02 per cent of the plant material.

The Fatty Oil.—This extract was a dark green semi-solid material. An attempt was made to determine the saponification value. Due to the intense green pigmentation it was impossible to get any values under ordinary circumstances. After trial it was found that relative values could be obtained using a spot plate and phenolphthalein as an indicator. In this manner the following values were obtained: 90.7; 93.5 and 90.7.

Three hundred Gm. of the fatty oil were saponified with alcoholic potassium hydroxide using a fifty per cent excess. The saponification was carried out in a reflux apparatus for one hour. The excess alcohol was then removed by distillation. One hundred and seven Gm. of non-saponifiable matter were obtained in this way.

The aqueous alkaline soap solution was now acidified with hydrochloric acid. The fats rose to the surface of the water. They were removed and washed with dilute sodium carbonate solution and then with water. In this manner 189 Gm. of fatty acids were obtained.

The Non-Saponifiable Matter.—The non-saponifiable matter obtained above was acetylated by boiling with acetic anhydride for one hour and then the mixture was poured into alcohol. The only product obtained was oily in character. No further study was made.

The Fatty Acids—Separation of the Solid from the Liquid Fatty Acids.—Modifying the Twitchell Method (1), 100 Gm. of the fatty acids were dissolved in 600 cc. of alcohol and the mixture heated to boiling. A boiling solution of 75 Gm. of lead acetate in 600 cc. of alcohol was added with constant stirring to the fatty acid solution. The resulting solution was cooled and allowed to stand at 0° for 12 hours. The solution was filtered at 0° and the filtrate is a solution of the lead salts (A).

The residue on the filter was boiled with 1200 cc. of alcohol and then cooled. This solution was set aside at 15° C. for 12 hours and filtered. The filtrate was reserved. The residue was treated again with boiling alcohol, set aside at 15° C. and filtered. The combined filtrates contained the lead salts (B).

The residue above was again boiled with alcohol and filtered while hot. The procedure was repeated. The filtrates containing the lead salts soluble in boiling alcohol were combined (C). The insoluble lead salts are (D).

The individual fractions above were freed of lead by treating with hydrogen sulfide in the alcoholic solutions and subsequent filtration to remove the lead sulfide. As explained above, the following fractions of the fatty acids were obtained:

A. Liquid fatty acids whose lead salts were soluble in alcohol at 0° C. Fatty acids with relatively high unsaturation, 35 Gm.

B. Liquid fatty acids whose lead salts are insoluble at 15° C. Fatty acids with relatively lower unsaturation than those above, 36 Gm.

C. Solid fatty acids whose lead salts are insoluble at 15° but are soluble at the boiling point of alcohol, 10 Gm.

D. Solid fatty acids whose lead salts are insoluble even in boiling alcohol, 20 Gm.

Bromination of the Liquid Fatty Acids.—Fractions A and B were in alcohol solution after the removal of the lead. The alcohol was removed by distillation in a stream of carbon dioxide. The acids were thrown into water, shaken out with ether and the ethereal solution, 250 cc. of

solution for both A and B were dried over anhydrous sodium sulphate. In the ethereal solution, the liquid fatty acids were brominated by adding chilled bromine dropwise to the chilled ethereal solutions. At no time did the temperature rise over -10° C. during the addition of the bromine. The temperature range was from 10° to -15° C. A fifty per cent excess of bromine was added in each case.

Linoleic Acid Hexabromide.—The precipitate which formed in the solution of (A) fatty acids was filtered off. No precipitate formed in the (B) fatty acids solution. The crystals obtained were washed and dried. They melted at $180-182^{\circ}$ C. Linoleic acid hexabromide melts at $180-182^{\circ}$ C. The crystals also contained 64.67 and 62.01 per cent bromine, respectively, according to two determinations. The theoretical bromine content of linoleic acid hexabromide is 63.32 per cent.

The ether was next removed by distillation from the fractions A and B. The residues were taken up in petroleum ether. A portion in each fraction remained insoluble. The melting points of these insoluble substances which were dark brown in color and not definitely crystalline were between 175° and 200° C. However, they contained only 7 to 17 per cent of bromine. No definite substances could be separated from these dark colored precipitates.

Linoleic Acid Tetrabromide.—After the removal of the above precipitates from the petroleum ether, there remained a solution of the petroleum-ether soluble fractions. After some evaporation and chilling for several days, fraction A yielded a precipitate of white crystals which weighed 0.4 Gm. and melted at $114-115^{\circ}$ C. Linoleic acid tetrabromide melts at $114-116^{\circ}$ C.

Strange New Acid Bromide.—Upon standing for a time after the separation of the linoleic acid bromide a further crystallization took place; 0.6 Gm. of a fine white crystalline powder was obtained. This substance melted sharply between 95° and 95.5° C. Recrystallization did not change the melting point. In addition to this the substance contained 46.33 and 46.94 per cent bromine, respectively, according to two determinations. In fatty acid literature there does not seem to be any product of a similar nature reported. Due to the small amount of the product it could not be further characterized. In 1930 Harwood (2) in his work on *Monarda punctata* seeds reported an acid whose bromide melted at 95° C. and which contained 46 per cent of bromine. These two products are possibly the same.

Oleic Acid Dibromide.—The petroleum ether was removed from the above solutions and a heavy reddish oil was obtained. Oleic acid dibromide is a liquid. The reddish oil was found to contain 35.03, 35.32 and 32.02 per cent of bromine, respectively, in three determinations. The theoretical bromine content of liquid oleic acid dibromide is 36.18 per cent. The yield was 31 Gm.

Solid Fatty Acids.—The alcohol was removed from the lead-free solutions of fraction C. This yielded 10 Gm. of fatty acids which were light green in color. The corresponding extract from fraction D weighed 20 Gm. and both fractions of the acids were dark green in color. No further information was obtained.

Water-Soluble Portion of the Alcoholic Extract.—The water-soluble portion previously deprived of its petroleum-ether-soluble material was first extracted with carbon tetrachloride. In preliminary tests it was found that warm carbon tetrachloride removed hydrothymoquinone in a condition that readily yielded to simple means of purification. The extract was a semisolid at room temperature but was fluid at the temperature of the water-bath.

Carbon Tetrachloride Extract.—The extraction was carried out in a flask provided with a mechanical stirrer and a reflux condenser. One liter of carbon tetrachloride was added to the extract in the flask and the whole was refluxed for one hour in a boiling water-bath with stirring. While still hot the underlying layer of carbon tetrachloride was poured off. All but 50 cc. of the solution was removed by distillation. The above procedure was repeated 6 to 7 times and the resulting extracts were combined.

Upon chilling the extracts, a greenish brown solid separated and was filtered off. The filtrate yielded nothing further even after treatment with charcoal.

Hydrothymoquinone.—The greenish brown solid obtained in the filtration above was placed in boiling water. The larger portion of the solid material went into solution and the resulting solution was filtered while hot. Upon cooling, hydrothymoquinone crystallized out spontaneously in a relatively pure condition. After repeated recrystallization from water a pure substance melting at $142-143^{\circ}$ C. was obtained. This substance gave the quinhydrone reaction

with thymoquinone. A mixed melting point with pure hydrothymoquinone was 142–143° C. and since the sample of hydrothymoquinone melted at 142–143° C. the substance was proved to be the quinone.

Pigments.—The residue insoluble in boiling water in the separation of the hydrothymoquinone was taken up in absolute alcohol. The material soluble in boiling absolute alcohol was obtained by filtering the hot solution. There was a small amount of yellow powder obtained after cooling, filtering the solution and drying. It melted at 216–218° C.

Upon cooling and partial evaporation of the alcoholic filtrate above, another yellow substance crystallized out. After repeated recrystallization from alcohol, yellow needles were obtained which melted at 204–205° C. The quantity was 0.2 Gm. This latter substance was soluble in ether, cold alcohol, water, cold carbon tetrachloride and chloroform. It gives a green coloration in alcoholic solution when treated with alcoholic ferric chloride. The reactions and properties of this compound strongly suggest the possibility that it is a pigment of the flavone group.

Ether Extract.—The aqueous extract was next extracted repeatedly with ethyl ether. Thirty-six Gm. of the ether-soluble extractive were obtained in this way.

SUMMARY AND CONCLUSIONS.

An alcoholic extract was prepared from the leaves and flowers of *Monarda menthaefolia*. An investigation into the constituents of this extract led to the isolation of the following:

- (1) A volatile oil
- (2) Non-saponifiable matter
- (3) Linoleic acid hexabromide
- (4) Linoleic acid tetrabromide
- (5) Unknown brominated acid melting at 95–95.5° C.
- (6) Oleic acid dibromide
- (7) Hydrothymoquinone
- (8) Solid fatty acids
- (9) Pigment melting at 216–218° C.
- (10) Pigment melting at 204–205° C.

PART II. ALCOHOLIC EXTRACTIVE OF MONARDA PUNCTATA var. LEUCANTHA.

During the autumn of 1933 a quantity of *Monarda punctata* var. *leucantha* was collected near Gainesville, Florida. The identification of the plant was confirmed by Professor Erdman West of the Agricultural Experiment Station, Gainesville, Florida.

The plant material, after comminution, was extracted with hot alcohol by decoction and subsequent expression in a lard press. After the removal of the excess alcohol there remained 1480 Gm. of extractive matter.

Petroleum-Ether-Soluble Extractive.—The extract above was extracted with petroleum ether (b. p. 60–70° C.). 300 Gm. of extractive were obtained after distilling off the excess petroleum ether.

The petroleum-ether extractive was next saponified using 50 Gm. of potassium hydroxide in two liters of alcohol. When saponification was complete, excess alcohol was removed by distillation and water was added to dissolve the soaps.

The Non-Saponifiable Matter.—The soap solution obtained above was now shaken with ether to remove the non-saponifiable material. After repeated shaking with ether, the ether solutions were united and dried over anhydrous sodium sulphate. The ether was removed by distillation.

The non-saponifiable matter was now steam distilled to obtain the volatile oil which is found here since the alcohols and hydrocarbons as well as the phenols, thymol and carvacrol, are soluble in ether and are extracted from alkaline solution by ether. In this manner 10 Gm. of oil were obtained.

Thirty Gm. of the non-saponifiable matter were acetylated by refluxing for two hours with acetic anhydride. When the acetylation was complete the mixture was poured into water, boiled and washed with warm water to remove the acetic anhydride.

After washing, the acetylated product was recrystallized a number of times from alcohol. In this way, several fractions were obtained which were the most soluble in alcohol and melted at approximately 90° C. There was also a fraction which melted at 110° C. The melting point of the latter fraction could not be raised by recrystallization.

Since sterols generally yield acetates melting higher than 110°, this fraction was saponified. The saponification value was 110. This would correspond to an alcohol with an atomic weight of 497 or about 33 carbon atoms. From the saponification mixture the alcohol or sterol was re-claimed and recrystallized. It melted at 68–70° C. (see Harwood (2)). The quantity of the material would not permit a thorough investigation into the properties of this substance but it is probable that the product in question is not a sterol but rather an alcohol with more than 30 carbon atoms.

By fractionally recrystallizing the acetates melting near 90° C., fractions were obtained which melted from 70° to 90° C. It is doubtful whether these are sterols or alcohols similar to that spoken of above.

Some of the products insoluble in alcohol were probably hydrocarbons.

The Saponifiable Matter—Fatty Acids.—After the removal of the non-saponifiable matter of the petroleum ether extract, the alkaline aqueous solution of the soaps was acidified with hydrochloric acid. The free fatty acids floated to the top of the liquid and were extracted with ether. The ether solution was dried over anhydrous sodium sulphate and then the ether was removed by distillation. In this way 180 Gm. of the fatty acids were obtained.

One hundred Gm. of the free fatty acids were dissolved in 750 cc. of alcohol in which 100 Gm. of lead acetate had been dissolved. The resulting solution was set aside in the cold at a temperature of 10° over night. The lead salts of the solid fatty acids, the precipitate, were removed by filtering and washed with cold alcohol. The liquid fatty acids were present in the filtrate as their lead salts.

The Solid Fatty Acids.—The lead soaps which were insoluble in alcohol at 10° C. were freed of lead by suspending in water acidulated with hydrochloric acid. The acid mixture was then shaken out with ether to remove the solid fatty acids. The resulting ether solution was dried over anhydrous sodium sulphate and then freed of ether by distillation.

In order to prepare the methyl esters of the fatty acids, the acids were dissolved in anhydrous methyl alcohol. Next the resulting solution was saturated with dry hydrochloric acid gas and refluxed for an hour. The resulting solution was poured into distilled water and the insoluble esters were washed until freed of acid, taken up in ether and dried over anhydrous sodium sulfate. The resulting methyl esters were fractionally distilled under reduced pressure and then the saponification value and melting point of the liberated acid obtained from each fraction were determined. The results are presented in the following table.

TABLE II.—THE SOLID FATTY ACID METHYL ESTERS.

	Fraction B. P.	Condition at Room Temperature.	Saponification Value.	M. P. of Free Acid.
1	110–165 at 25 mm.	Liquid	206	51
2	165–195 at 18 mm.	Liquid	206	50–51
3	195–200 at 15 mm.	Liquid	197	55
4	200–215 at 15 mm.	Semisolid	191	47
5	215–230 at 15 mm.	Solid	174	52
6	230 up at 15 mm.	Solidified in condenser	137	51–52
7	Residue in flask soluble in alcohol	Solid	100	69–70

From the data obtained on the foregoing methyl esters it appears that the fractions 1, 2 and 3 contain a mixture of varying percentages of myristic, palmitic and stearic acids. Fraction 4 is probably made up mostly of palmitic and stearic

acids while fractions 5 and 6 contain principally stearic and arachidic acids. From the saponification value and higher melting point of fraction 7 there may be solid fatty acids as high or higher than arachidic acid in the mixture.

Liquid Fatty Acids.—The liquid acids obtained above in ether solution were chilled to 0° C. after drying. Then a solution of bromine in acetic acid at 0° C. was run in dropwise with stirring until an excess of bromine was present. The resulting solution was set aside in an ice box over night whereupon a precipitate formed. The insoluble crystals were filtered off, washed with ether and dried. They melted at 180–181° C. Linoleic acid hexabromide melts at 180–182° C.

The ethyl ether was removed from the brominated fatty acids and then an equal quantity of petroleum ether was added to the residue. A semisolid portion separated out on the bottom of the container and this was removed and later a second deposit was also removed. These substances melted at a temperature of 134–136° C. This is unusual because the customary unsaturated acids have never yielded a bromide melting in that range according to reports. However, there is a reported bromide from the fats of chaulmoogra oil which melts within this range. The bromine content of the samples was not determined due to lack of material.

The solution after the removal of the above substances was freed of petroleum ether by distillation. The resulting red oil weighed 48 Gm. It is oleic acid dibromide.

Ether Extract.—After the removal of the petroleum-ether-soluble portion of the alcoholic extract, ethyl ether was used to extract the residue. By repeated extraction, 130 Gm. of the extract was obtained. The greatest part of this latter extract was soluble in carbon tetrachloride.

Since hydrothymoquinone has been found in the extracts it was possible that it might be found here. Consequently, the extract was boiled in water and the hot water solution was filtered boiling hot. It was found that there was no hydrothymoquinone present. The above treatment rendered the ether extract into a water-soluble and water-insoluble portion.

The water-soluble portion of the extract turns cherry red when treated with potassium hydroxide solution, and when the alkaline solution is heated a small amount of ammonia gas is given off. The original solution was distinctly acid to litmus.

Lead acetate was used to precipitate the aqueous solution. After the precipitation the lead was removed from the solution by passing hydrogen sulfide through it. The precipitate was also freed of lead by suspending in water and passing hydrogen sulphide through the suspension. After filtering the above two mixtures to remove the lead sulphide, the resulting solutions were evaporated to dryness. The solutions probably contained tannins and their derivatives because the resulting products gave a green color with ferric chloride. Otherwise, there was no satisfactory evidence inasmuch as these products were semisolids and could not be crystallized.

No tests for glucosides were obtained by using hydrolysis and reducing tests.

Chloroform Extract.—The residue remaining after the ethyl ether extraction was extracted with chloroform; 150 Gm. were obtained but nothing was separated from the extract.

Ethyl Acetate Extract.—After the chloroform extraction was completed, an attempt was made to extract the residue with carbon tetrachloride. The extract was so small that it was added back to the residue and the whole was then extracted with hot ethyl acetate. Upon evaporation and cooling of the ethyl acetate solutions, a white precipitate formed. By repeated evaporation and crystallization, 25 Gm. of the substance was obtained.

The substance above was insoluble in hot and cold water, soluble in alcohol and insoluble in ether. In attempting to run a melting point it blackened at 250–260° C.

No further information was obtained concerning the alcoholic extractive.

SUMMARY AND CONCLUSIONS.

An alcoholic extract was prepared from the entire plant of *Monarda punctata* var. *leucantha*. After investigating, the following substances were found to be present:

- (1) A volatile oil
- (2) Possibly sterols
- (3) High alcohols
- (4) Hydrocarbons

- (5) Probably the following fatty acids:
- (a) Myristic
 - (b) Palmitic
 - (c) Stearic
 - (d) Higher acids including arachidic
- (6) Linolenic acids
- (7) An unsaturated fatty acid whose bromide melts at 134–136° C.
- (8) Oleic acid
- (9) A product from ethyl acetate extract that has not been characterized
- (10) No lecithin
- (11) No hydrothymoquinone.

PART III. MISCELLANEOUS DETERMINATIONS.

As a preliminary study *Monarda menthæfolia* plant parts were submitted to the Dragendorf selective solvent analysis. As a result the following results were obtained.

TABLE III.—SELECTIVE SOLVENT DETERMINATIONS, MONARDA MENTHÆFOLIA.

Determination.	Flower Heads.	Leaves.	Stems.
Petroleum Ether	4.27	5.68	2.70
Ethyl Ether	2.11	2.22	0.55
Chloroform	0.43	1.09	1.11
Alcohol	24.76	16.17	11.26
Water	9.63	12.37	3.00
Aqueous KOH (2%)	26.59	30.36	35.73
Aqueous HCl (1%)	5.55	8.66	10.44
Dregs	16.65	13.16	25.05
Moisture	10.57	10.21	9.11
Total	100.56	99.92	98.95

NOTE: Percentage results by averaging two determinations.

Also in connection with the present investigation some determinations on structural elements were run. These determinations include:

1. The A. O. A. C. Pentosan determination as recommended by the Association of Plant Physiologists (3).
2. The A. O. A. C. Tannin determination (4).
3. The U. S. P. X Crude Fiber Determination.
4. The Crude Fiber Determination, the so-called "Dutch Method" of Wallis and Goldberg (5).

The crude fiber determinations are empirical and results are dependent upon the method used.

Results obtained are tabulated in Tables IV and V.

TABLE IV.—MISCELLANEOUS DETERMINATIONS, MONARDA MENTHÆFOLIA.

Determinations.	Flower Heads.	Leaves.	Stems.	Roots.
Pentosan	10.09	8.59	17.42	16.44
Crude Fiber				
U. S. P.	12.65	10.62	42.32	35.74
Dutch Method	10.43	9.20	28.69	23.52
Tannin	2.90	4.85	3.89	6.30

TABLE V.—MISCELLANEOUS DETERMINATIONS, *MONARDA PECTINATA*.

Determination.	Flower Heads.	Leaves.	Stems.	Roots.
Pentosan	11.98	17.10	25.16	30.00
Crude Fiber				
U. S. P. X	26.96	15.32	45.74	47.03
Dutch Method	16.77	13.64	26.77	29.18
Tannin	1.23	3.11	2.87	2.39

SUMMARY AND CONCLUSIONS.

The results of preliminary examinations and standard tests for the customary plant constituents are tabulated. The Dragendorff determinations are run on the leaves, stems and flower heads of *Monarda menthæfolia* and the pentosan, crude fiber and tannin determinations are run on the parts of the plant of both *Monarda menthæfolia* and *Monarda pectinata*. The Dutch Method for the determination of crude fiber gives consistently lower results than the official U. S. P. method.

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SELENIUM DISTRIBUTION IN AND SEASONAL VARIATION OF TYPE VEGETATION OCCURRING ON SELENIFEROUS SOILS.*¹BY O. A. BEATH,² H. F. EPPSON AND C. S. GILBERT.

In previous references (1, 2, 3) relating to the occurrence of selenium in native range plants no significant data were given as to its distribution in the plant itself nor was there offered at that time or since definite experimental evidence as to fluctuations of selenium with seasonal growth and development. This presentation, therefore, is an attempt to show that the amount of selenium in a seleniferous plant is not constant nor is its distribution in the plant uniform in any one part during a growing season. Many qualifying statements are necessary in attempting to show the quantitative assignment of selenium in the native range plants as they exist under natural conditions.

Geological Correlations.—The authors (1, 2, 3) in earlier publications have advanced experimental evidence to show that some native range plants, not necessarily generically related, absorb selenium in varying amounts when occurring upon shales and soils of a definite geological classification. These formations outcrop in many sections of the Rocky Mountain region. At the present time these include rocks of Cretaceous and Eocene ages and in addition two strata in the Chugwater formation. The latter is whole or in part Permian or Triassic (?) age. The authors have stressed the fact that in dealing with seleniferous plants there are a few that stand out in their normal affinity for selenium. They constitute, therefore, a major group (Fig. 1) of positive selenium carriers. A seleniferous plant, for example, *Oenopsis condensata* will yield a comparatively low selenium value when collected on any member of the Benton, Mesaverde or Ferris

* Presented before Scientific Section, A. PH. A., Dallas meeting, 1936.

¹ Paper presented before Botany Section of the Southwest Division of the American Association for the Advancement of Science, Flagstaff, Arizona, April 27, 1936.² Professor of Research Chemistry, University of Wyoming, Laramie, Wyoming.