(4) Hodgson, *Am. J. Pharm.*, 273 (1832).
(5) Russell, *Ibid.*, 90, 8–15 (1918).
(6) Dohme and Kelly, JOUR. A. PH. A., 52, 561 (1904).
(7) Chiani, La Clin. Ocul. 11 (1911); *Zentr. Biochem. Biophys.*, 12, 557 (1911–1912).
(8) Lohmann, JOUR. A. PH. A., 51, 317–319 (1903).
(9) Podwyssotski, V., *Pharm. J.*, III, 12, 217, 1011 (1881–1882); *Arch. Pharm. Exper. Pathol.*, 13, 29 (1880); *Pharm. Z. Ruszland*, 12, 44 (1881); *Ber.*, 15, 377 (1882).
(10) Disque, Ludwig, *Sitz. Abhandl. naturforsch. Ges. Rostock*, N. F. 5, 1–35 (1913).
(11) Munch, James C., *Bioassays*, 772 (1931).
(12) Kuersten, *Arch. Pharm.*, 229, 220–248 (1891).
(13) Dunstan and Henry, *J. Chem. Soc., Trans.*, 73, 209–226 (1898).
(14) Borsche and Nieman, *Ann.*, 494, 126 (1932); *Ibid.*, 499, 59 (1932); *Ber.*, 65B, 1633 (1932); *Ibid.*, 65B, 1846 (1932).
(15) Spathe, Wessely and Kornfeld, *Ibid.*, 65B, 1536 (1932); *Ibid.*, 65B, 1846 (1932).

A PHARMACOGNOSTIC STUDY OF *CHRYSANTHEMUM BALSAMITA* L., VAR. *TANACETOIDES* BOISS., TOGETHER WITH A STUDY OF ITS VOLATILE OIL.*,[1]

BY RALPH F. VOIGT, CHARLES H. ROGERS AND EARL B. FISCHER.[2]

The overground parts of *Chrysanthemum Balsamita* L., var. *tanacetoides* Boiss. (Family, *Compositæ*), contain a volatile oil, the odor of which is almost indistinguishable from that of oil of Spearmint. The striking similarity of the two oils, together with the fact that the plants yielding them come from different families, induced us to seek information in the literature concerning the composition of the oil of *Chrysanthemum Balsamita* L., var. *tanacetoides* Boiss. as compared to that of Spearmint. Very few references were found reporting on the chemistry of the oil, the ones that are available giving information only on physical constants. A number of references of a botanical nature are recorded and a few of historical interest.

The purpose of this study was to investigate in part the chemistry of the volatile oil, to determine the yields at various seasons of the year, and to study the morphology and histology of the plant. Material employed was obtained from plants collected in Michigan, Wisconsin and Minnesota and cultivated in the Medicinal Plant Garden of the College of Pharmacy, University of Minnesota.

Chrysanthemum Balsamita L., var *tanacetoides* Boiss. is an herbaceous, woody perennial native to western Asia. It was introduced into Europe and from there found its way to the United States. It is commonly known as Costmary, Old Maid, Sweet Susan, Sweet Mary and Mint Geranium. History reveals that the plant has been used in the treatment of many common ailments. In the British Pharmacopœia of 1788 it was recognized and used as an aperient. Some of its other uses have been as a diuretic, astringent, antiseptic, stomachic and anthelmintic.

PHARMACOGNOSTIC STUDY—EXTERNAL MORPHOLOGY.

Flower.—The florets are assembled into small closed heads, which form a corymbose inflorescence. The individual heads vary up to 8 mm. in diameter. They appear cup-shaped

* Presented before the Scientific Section, A. PH. A., New York meeting, 1937.

[1] Abstracted from a thesis submitted to the Graduate School of the University of Minnesota by Ralph F. Voigt in partial fulfilment of the requirements for the degree of Master of Science, June 1937.

[2] University of Minnesota, Department of Pharmacognosy, College of Pharmacy, Minneapolis.

and flattened on top. The involucre consists of more or less dry and scarious imbricated bracts which give the involucre a pale green color. The head is discoid and homogamous, consisting only of tubular florets, the number of which is variable. The individual florets vary up to 3 or 4 mm. long and 0.5 mm. wide. The corolla is tubular and regularly 5-lobed with veins bordering on the margins of the lobes. In place of a pappus there is only a ridge or swelling at the base of the corolla. The pistil consists of an inferior ovary which is developed into an achene. The ovary and consequently the achene is formed by the union of two carpels. The achenes are located upon a naked, flattened receptacle. The wall of the achene is 5 to 10 nerved and contains a single erect anatropous seed which arises slightly to one side of the middle of the base. The style portion of the pistil has a bulb-like enlargement just above its base and is more or less deeply bifid at the apex with the stigmatic surfaces in two lateral lines.

There are 5 stamens attached alternately with the petals. The stamens are structurally uniform and their filaments are inserted on the tube of the corolla. The filaments are narrowly band-shaped and free, and the anthers 2-celled, rounded at the base and fused together to form a tube.

Leaves.—The basal leaves are from 20 to 38 cm. in length and approximately 5 to 8 cm. broad, the petioles constituting approximately half the length. The lamina is oblong to elliptical with a rounded apex and usually a somewhat unequal tapering to rounded base. The margin is crenate-dentate. The upper surface is green and slightly puberulent. The lower surface is a lighter green and puberulent to glaucous. The texture is membranaceous or herbaceous. The midrib of the leaf is more prominent on the lower surface and the venation is pinnate-reticulate. The petiole is distinctly triangular in cross section and 3-ribbed with one surface corresponding to the lamina of the leaf.

The cauline leaves are in a two-fifth arrangement upon the stem. As they ascend the stem, their petioles become shorter to a point approximately three-fourths the distance from base to the apex where the leaves are sessile. The shapes of the lower ones are ovate to broadly elliptical while some of the upper ones may be oblong and others oblanceolate. The apexes are somewhat acute rather than being rounded as is the case with the basal leaves, and the tapering bases of the laminæ tend to wing down along side the midrib or petiole and connect with the lower lobes, of which latter the number may vary from two to five or six. The smaller lobes are closer to the place of attachment. The greater number of stipules are on the lower leaves while two are commonly found on the upper leaves. Some of the leaves show a tendency toward lobing by being deeply indented at the base of the lamina. The other features of the cauline leaves correspond to those of the basal leaves.

Stem.—The stem is an erect, cylindrical axis extending in length from 60 to 120 cm. and varying from 4 to 9 mm. in diameter at the base. It is green and nearly glabrous, longitudinally ribbed with three ribs for each leaf, which arise at a point somewhat below each leaf and gradually become very prominent as they near the base of the leaf. Two of these ribs come in direct line with the extreme borders of the leaf at the place of attachment and one runs directly into the middle of the leaf and becomes a part of the petiole of the leaf or of the lamina if the leaf is sessile. The internodes are from 1.4 to 4.8 cm. in length. Short lateral leafy branches arise in the axils of the leaves near the base of the stem while higher up longer leafy flowering branches may develop and these in turn may give rise to a few shorter flowering branches. The fracture of the stem is hard, rough and woody, and there are a few scattered brown spots on the stem surface near the base.

Rhizome.—The rhizome is vertical to oblique with horizontal branches extending laterally. These rhizomes are cylindrical and vary from 3.5 to 15 cm. in length and 3 to 12 mm. in diameter. The vertical portions are thickly covered with adventitious roots. They are of a very dark brown or almost black color, and the surfaces are more or less irregularly roughened. The thickness of the vertical rhizomes is from two to three times that of the horizontal rhizomes that arise from them. The horizontal rhizomes lie more or less close to the surface of the ground and may or may not be branched. They are marked with several buds or stem scars on the upper surface and an occasional node. The nodes are more numerous toward the end at which large numbers of buds and leaves tend to reach above the surface. These rhizomes are of a medium to a dark brown color and have surfaces which are smooth in some places or roughened elsewhere. The fracture

Plate No. 1
CHRYSANTHEMUM BALSAMITA L.,
VAR. TANACETOIDES BOISS.
Flowering Stem

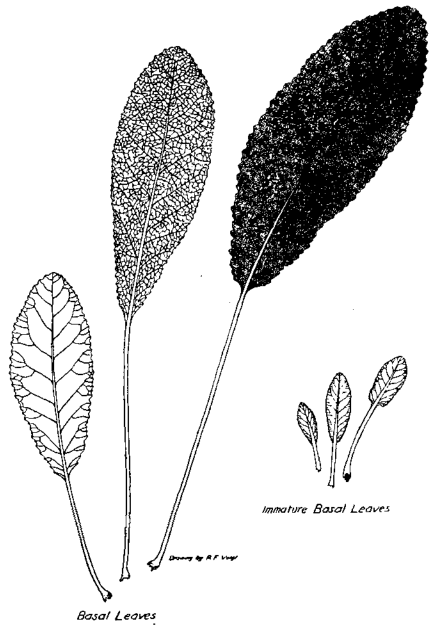

Plate No. 3
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.

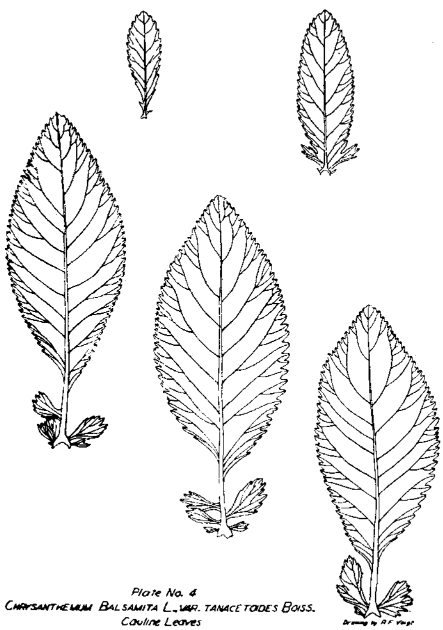

Plate No. 4
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Cauline Leaves

Plate No. 5
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Root System

is very hard, rough and woody. From the lateral and lower surfaces, which are marked with many root scars, arise many long cylindrical fibrous roots.

Roots.—The roots are fibrous and may be up to 30 cm. in length and 2 mm. in diameter. They are evenly cylindrical, filiform, more or less curved and tortuous. The surface is uniformly smooth and of a dark brown color. There are a few rootlet scars. Only a few branches occur but numerous rootlets arise near the end.

HISTOLOGY.

Petiole and Leaf.—The epidermis of the petiole consists of uniformly rectangular cells regularly arranged with a few stomata. Just beneath the epidermis on the right and left abaxial sides is a considerable area of parenchyma with large elongated intercellular spaces. The tissue beneath the epidermis on the adaxial side also shows some parenchyma with intercellular spaces. In the upper right and left corners and in the abaxial corner, just beneath the epidermis, are small areas of collenchymatous cells. These cells possess characteristic abnormal thickenings at the corners. Thin-walled parenchyma varying up to about 0.100 mm. in diameter with intercellular spaces between them make up the major portion of the petiole. Near the surface there are occasional schizogenous ducts which run longitudinally. In each of the upper right and left corners is a vascular bundle and a large bundle is located in the lower central portion. The bundles are of the collateral type and well developed with prominent secondary tissues. On either side of the central vascular bundle are smaller vascular bundles consisting largely of primary xylem and phloem with a slight secondary development.

In the transverse section of the midrib the epidermis is similar to that of the petiole. The tissue of the midrib is made up principally of spongy parenchyma which extend a short way into the lamina. On the adaxial side, the palisade cells of the lamina extend into the midrib and show a gradual transition to the polygonal parenchyma cells. There is a little collenchymatous tissue in the lower corner. The thin-walled parenchyma cells are very similar to those of the petiole. The main vascular bundle, which is similar to that of the petiole, is centrally located.

In the transverse section of the leaf the tissue consists of an upper and lower epidermis made up of irregularly rectangular cells among which are interspersed at more or less regular intervals, numerous stomata. Between the upper and lower epidermal layers the leaf tissue consists of very loosely arranged cells, some of which resemble palisade cells, others resembling the usual type of mesophyll cell. Very large intercellular spaces are to be observed in the central portions of the lamina and at more or less regular intervals small vascular bundles are located. The contents of the epidermal cells and the cells of the spongy layer consist largely of protoplasmic material. On both upper and lower surfaces the epidermal cells are frequently modified into glandular and non-glandular hairs. Descriptions of the structure of these cells is included in a succeeding paragraph.

In the surface view of the upper and lower epidermal cells the vertical walls are thin, very wavy, and a certain uniformity in the shape of these cells is to be observed. Broadly elliptical stomata from 0.034 to 0.043 mm. in length are distributed among the epidermal cells of both lower and upper epidermis. The variation in size of the stomata of both upper and lower epidermis is confined to the same limits. In the upper epidermis each stoma is bordered usually by five epidermal cells, occasionally by only four, while in the lower epidermis the usual number of cells bordering each stomata is four, occasionally three or five. A greater number of stomata are to be found among the lower epidermal cells.

The T-shaped non-glandular hairs usually have a 3-celled stalk with a unicellular terminal portion running at right angles to the stalk and having approximately equal arms. The length of the apical portion of these non-glandular hairs varies up to 0.459 mm. and the diameter is about 0.043 mm.

The glandular hairs are usually situated in a depression in the leaf surface. The arrangement of the epidermal cells at the points where these hairs are attached is quite characteristic and typical. In side view, these hairs are seen to consist of two rows of cells about four deep. A thin cuticle encloses the entire hair. As seen from above, these hairs appear to be broadly elliptical and vary in size according to their content of volatile oil. The length varies from 0.032 to 0.098 mm. and the width from 0.032 to 0.073 mm. The number of these hairs is greater on the lower surfaces of the leaves.

Stem.—The epidermal layer consists of small rectangular cells which are elongated tangentially. Immediately beneath the stem epidermis is a hypodermal layer composed of nearly square cells, the outer walls of which are considerably thicker than the inner and radial walls. Beneath the hypodermis is a layer of cortical parenchyma approximately ten cells in thickness, the parenchyma cells of this region being more or less elliptical and having distinct intercellular spaces of varying size between them. The cell walls of the cortical parenchyma consist of cellulose. Regularly distributed through the cortex just beneath the hypodermis and at places where ridges or ribs run longitudinally along the stem, occur areas of collenchyma cells. These areas in transverse sections are triangular in shape. Also through the cortical parenchyma there are occasionally distributed thick-walled stone cells which may occur singly or in small groups. In transverse sections these stone cells are oval in shape whereas in logitudinal sections they are rectangular. They correspond in size very closely to the parenchyma cells. The walls of the stone cells are lignified, lamellated and penetrated by many simple pores.

The endodermis consists of a single uninterrupted layer of tangentially elongated cells, the cells being larger in those portions of the endodermis which are located over the regularly distributed bundles of pericyclic fibers. The cells of the endodermis are thin-walled and non-lignified.

Centrifugal to the secondary xylem and within the endodermis are groups of heavy-walled fibers associated with sieve tubes, companion cells and a few thin-walled fibers. The dome-shaped masses of heavy-walled fibers are those of the pericycle, while the sieve tubes, companion cells and thin-walled fibers constitute the phloem, the most centrifugally located of these latter elements representing primary phloem. Alternating with these complex groups are largely parenchymatous regions which, in the portion or vicinity once occupied by cambial cells, may show some sieve-tubes and companion cells. The latter two types of cells, possibly in addition to parenchyma cells in the immediate area, represent secondary phloem while the balance of this tissue (the more centrifugal parenchyma) represent the parenchymatous portion of the pericyclic region.

The secondary xylem consists principally of thick-walled heavily lignified fibers with numerous tracheæ scattered throughout. The arrangement of fibers, tracheæ, wood fibers and tracheids in the secondary xylem is in definite rows which extend radially in the stem. Within the secondary xylem is a relatively narrow zone of primary xylem composed also of fibers and tracheæ, both of which are strongly lignified. This may be followed by the medullary sheath consisting of only a few cells. Making up the central portion of the stem are numerous thin-walled pith cells. In transverse view the cells are spherical with large intercellular spaces, while longitudinally the cells are rectangular.

In the autumnal stem the cambium layer is wanting. The secondary xylem joins up closely to the secondary phloem and forms a continuous band around the stem. The medullary ray cells are also wanting. That separated vascular bundles once existed in the younger stems is indicated by the more or less wedge-shaped extensions of the primary xylem into the pith area in a direct radial line with the primary and secondary phloem.

Rhizome.—Transverse and longitudinal sections of the rhizome show either an epidermal layer, or an outer corky layer which represents the periderm. The periderm consists of several layers of phellogen immediately within a single layer of cork cells, the structure of which latter cannot be easily determined. The walls of the cells constituting the outer cork layer are dark brown in color. The epidermal cells, where present, are rectangular and tangentially elongated. Hypodermis occurs wherever the epidermis is present and is distinct and composed of rectangular cells.

The cortical tissue within the epidermis or periderm, as the case may be, is composed of thin-walled oval cells and between these occur large intercellular spaces. The cortical parenchyma seem to be arranged in concentric rows around the rhizome. This regular arrangement is noticeable only in the outer portion of the cortex and gradually disappears toward the inner portion near the endodermis where the cells become irregular in shape, size and position. In tangential and radial longitudinal sections the cortical parenchyma are rectangular in shape. Occasional, solitary stone cells are distributed through the cortex. These present an oval shape in transverse section and are rectangular in the longitudinal direction. Their walls are somewhat thickened, lamellated, heavily lignified, and possess many simple pores. Scattered through the cortex also are a number of small schizogenous cavities. These cavities are elongated in a longitudinal direction in the rhizome. The endodermis consists of a single row of cells that

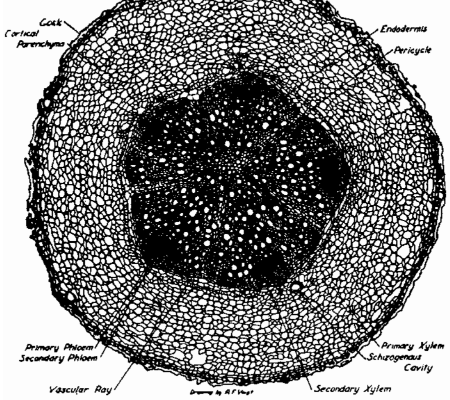

Plate No. 18
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Transverse Section of Root

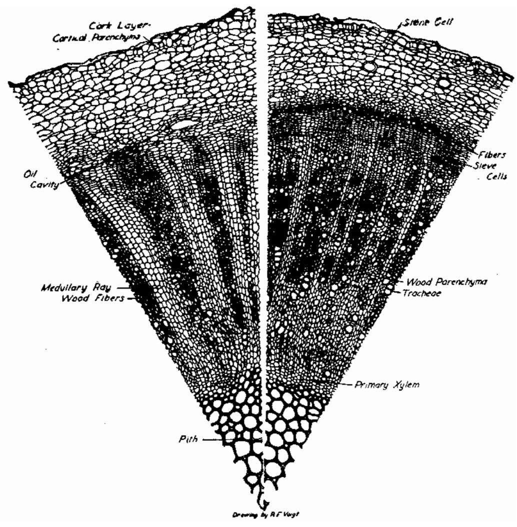

Plate No. 15
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Transverse Section of Rhizome

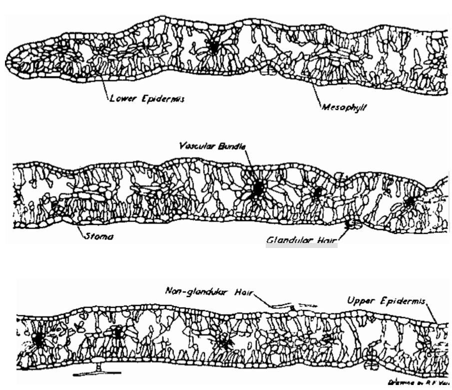

Plate No. 8
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Transverse Sections of Leaf

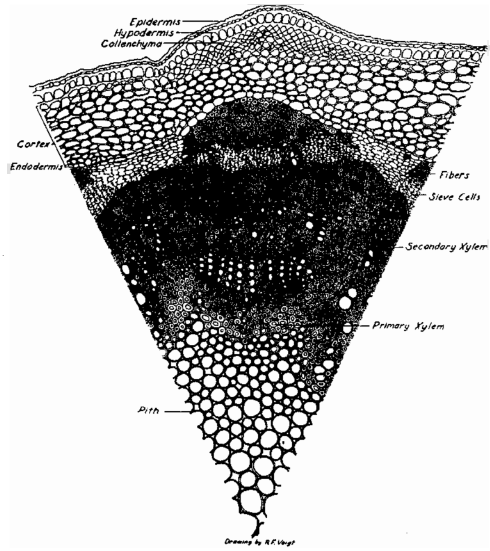

Plate No. 12
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Transverse Section of Stem

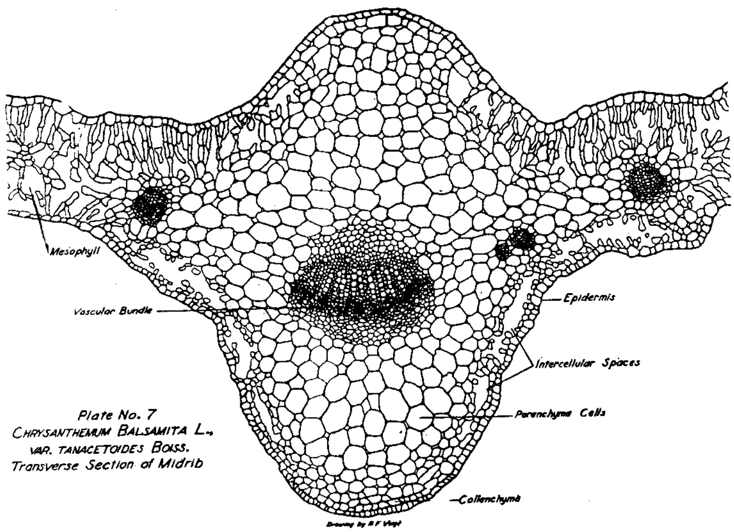

Plate No. 7
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Transverse Section of Midrib

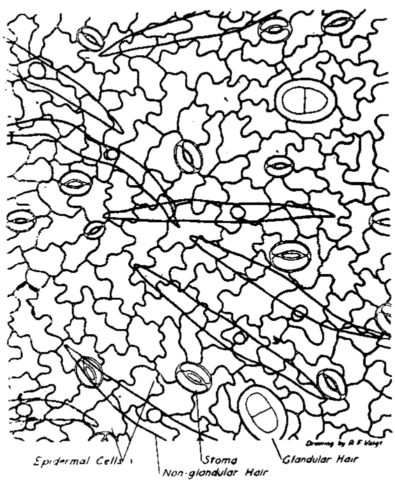

Plate No. 10
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Lower Leaf Epidermis

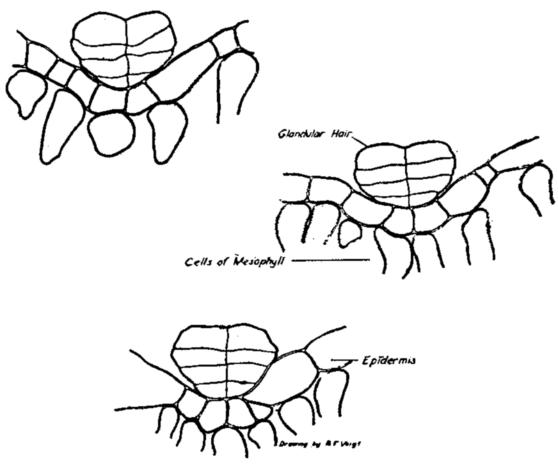

Plate No. 11
CHRYSANTHEMUM BALSAMITA L., VAR. TANACETOIDES BOISS.
Showing Structure and Attachment of Glandular Hairs

are distinct in places but become indistinct or almost obliterated in others. Small flat groups of fibers occur intermittently beneath the endodermis. These bundles constitute the primary phloem and the number of fibers contained in each is variable. The individual fibers have thinner walls than those of the primary stem phloem. A secondary phloem wedge occurs directly beneath each primary phloem wedge and is separated from the secondary xylem within by a definite cambium layer. The secondary xylem is arranged in regularly spaced radial wedges separated from one another by distinct medullary rays which are from two to six rows wide. In the secondary xylem wedge are found numerous vessels, singly or in groups of two or three. These vessels are mostly of the bordered pit type with an occasional reticulate or spiral vessel. Distributed among the vessels are wood parenchyma and wood fibers. The medullary rays vary from two to six rows wide. In the xylem region the cells as seen in transverse section are rectangular being slightly elongated in a radial direction. Outward toward the phloem the xylem cells become more nearly square and then distinctly elongated in a tangential direction in the region near the endodermis. From the cambium outward the rays increase in width. At the interior of each secondary xylem wedge is a small area of primary xylem, consisting almost entirely of tracheæ and wood parenchyma.

Finally, the pith which is composed of cylindrical cells occupies the central portion of the rhizome. The cells have small intercellular spaces. The walls are fairly thick and possess many simple pits.

Root.—In place of the epidermis there is in all the sections examined a thin outer layer of cork, the outer layers of which consist of more or less obliterated cells with about two inner rows of tabularly arranged cells. The latter cells probably constitute the phellogen. Immediately beneath the phellogen layers and for a distance approximately one-half way into the root section extends the cortical parenchyma layer. This layer consists of nearly isodiametric cells, the shape of the individual cells being oval to irregularly polygonal and the walls consisting of cellulose. Many of the oval cells are divided into two, three or sometimes four parts by transverse walls which are somewhat thinner than the surroundings walls. The parenchyma cells of this cortical layer are elongated or oblong in logitudinal view. Large intercellular spaces are distributed frequently through the cortex and near the endodermis and radially in a line with the primary phloem wedges occur a few schizogenous cavities. The endodermis is distinct and consists of a single layer of rectangular non-lignified cells. The endodermal layer is continuous except at a few points where the primary phloem has been pushed out by the development of secondary phloem and xylem. A definite pericycle occurs within the endodermis and is represented by a zone of parenchyma cells one to two cells in width. In the transverse sections of roots examined there occur five wedges of xylem and phloem, the wedges separated from one another by narrow vascular rays. A small primary phloem occurs adjacent to the pericycle at the outer limits of each phloem-xylem wedge. A distinct cambium is present between secondary phloem and secondary xylem. The secondary xylem consists of tracheæ of the bordered pit type with tracheids, wood parenchyma and lignified fibers. The primary xylem occupies a small area at the center of the root. Typically it is pentarch.

STUDY OF THE VOLATILE OIL.

For purposes of this study, the volatile oils were obtained by subjecting the fresh or partially dried overground parts to steam distillation and since the yield of oil proved to be rather low the distillate was cohobated to avoid loss of oil by solution and the cohobated oil combined with that separated mechanically from the distillate.

DESCRIPTION OF SAMPLES AND YIELDS OF OIL.

Sample No. 1. July 1930, material collected in Michigan, partially dried, yielded 0.09 per cent of oil. The non-rectified oil was light amber in color and the odor and taste similar to Spearmint. Tests were made July 15, 1932.

The remaining samples were steam distilled from leaves and tops of plants grown in the University of Minnesota Medical Plant Garden.

Sample No. 2. Fresh material distilled July 9, 1931, yielded 0.08 per cent of oil. When rectified the yield was 0.064 per cent. The rectified oil possessed a golden-yellow color. Tests were made July 15, 1932.

Sample No. 3. Fresh material distilled October 22, 1931, yielded 0.046 per cent of oil. Tests were made July 15, 1932.

Sample No. 4. Fresh material distilled July 8, 1932, yielded 0.056 per cent of oil. Tests were made July 15, 1932.

Sample No. 5. Fresh material distilled June 26, 1933, yielded 0.071 per cent of oil.

Sample No. 6. June 29, 1933, material dried at room temperature yielded 0.121 and 0.644 per cent of oil, basis of fresh and dried material, respectively.

Sample No. 7. October 18, 1933, material dried at room temperature yielded 0.065 and 0.38 per cent of oil, basis of fresh and dried material, respectively.

Sample No. 8. July 2, 1934, material dried at room temperature yielded 0.118 and 0.588 per cent of oil, basis of fresh and dried material, respectively.

Sample No. 9. July 23, 1935, material dried at room temperature yielded 0.096 and 0.490 per cent of oil, basis of fresh and dried material, respectively.

TABLE I.—ANALYTICAL RESULTS.

Oil.	Specific Gravity 25° C.	Refractive Index 20° C.	Specific Rotation at 25° C.	Percentage Carvone.*
Sample No. 1	0.9473	1.4892	−44.21°	69.6
Sample No. 2	0.9762	1.4970	−40.10°	81.8
Sample No. 3	0.9837	1.4887	−37.92°	71.4
Sample No. 4	0.9580	1.4974	−50.63°	73.8

* To determine the per cent of carvone in the oil, the method as outlined in the U. S. P. X (1) and described by Jenkins and DuMez (2) was followed.

Carvone.—The aqueous solutions from the carvone determinations were combined and treated with sodium hydroxide solution (10 per cent) to free the carvone from its sulfite addition product. The carvone was removed from this mixture by steam distillation and was obtained in the form of a colorless clear liquid.

The physical constants of the extracted carvone are as follows:

Specific gravity at 15° C.	0.9669
Refractive index at 20° C.	1.4990
Specific rotation at 20° C.	−59.20°

Carvoxime.—The carvoxime was prepared by treating a portion of the oil or a portion of the purified carvone as obtained above, in alcoholic solution, with hydroxylamine hydrochloride and sodium bicarbonate. After refluxing for 15–20 minutes and cooling, water was added, the alcohol distilled from the mixture and the residue distilled with steam. The carvoxime crystals separating from the aqueous distillate were filtered off and recrystallized from absolute alcohol. A white crystalline compound was obtained.

The melting point of the carvoxime from the oil of this Chrysanthemum species was 72° C. This represented the average of the melting points of four separate samples of carvoxime. Determinations could be easily duplicated to within one-half degree.

The melting point of carvoxime which was prepared from oil of Mentha Viridis, in a similar manner, was also determined as 72° C.

As a final check, the carvoxime from the Chrysanthemum species was mixed with the carvoxime from Spearmint and the melting point of the mixture was determined as 72° C.

2,4-Dinitrophenylhydrazone.—The dinitrophenylhydrazone crystals were prepared according to the method described by Kamm (3).

The melting point of 2,4-dinitrophenylhydrazone of oil of Chrysanthemum species was 186° to 187.5° C.

DISCUSSION.

In the morphological examinations it has been found that the description of the plant as published in the botanical manuals such as Gray, and Britton and Brown are essentially correct. Only one point of difference is to be noted and that is in connection with the nature of the cauline leaves. Gray described the cauline leaves as often possessing two small lateral lobes at their bases. This examination shows that there may be as many as five or even six lateral lobes present on some cauline leaves. The usual number, however, is four.

It has also been noted that two plants which differ distinctly from each other in certain floral characteristics have been named in botanical texts under the same species. The difference is indicated only as a varietal one. One of these plants, the *Chrysanthemum Balsamita* L. concerned in this study is characterized by always possessing flowers which are rayless. Occasion has arisen to examine the other plant known by the same botanical name, and the examination has shown that the flowers in this case always possess short white rays. A distinct chemical difference is also to be noted, however, between the constituents of these two plants. In the work described in this thesis, the plant with the rayless flowers has been shown to possess a volatile oil, the chief constituent of which has been definitely identified as carvone, whereas the plant possessing flowers with short white rays is known to possess a volatile oil the principle constituent of which, is a white crystalline solid, the characteristics of which are similar to those of camphor, borneol or some similar substance. Although further chemical investigation of the oils produced by these two plants will be carried on, it would seem that the morphological differences in the flowers in addition to the distinct difference in chemical composition of their oils would warrant the placing of one or the other of these plants in a separate species.

The comparison between yields, physical constants and percentage carvone content of the oils of Costmary and Spearmint are shown by the following table:

Table II.

	Oil of *Chrysanthemum Balsamita* var. *tanacetoides*.	Oil of Spearmint.
Yield of oil (per cent)	0.046 to 0.121	0.3 to 1.5
Specific gravity at 25° C.	0.9473 to 0.9837	0.917 to 0.934*
Refractive index at 20° C.	1.4887 to 1.4974	1.4820 to 1.4900*
Specific rotation at 25° C.	−37.92° to −50.63°	−46° to −59°*
Carvone content (per cent)	69.6 to 81.8	35 to 66

* U. S. P. XI.

The specific gravity of the oil of Chrysanthemum is considerably higher than that of oil of Spearmint and the refractive index of the former is slightly higher than that of Spearmint. The optical rotation of the Chrysanthemum oil is slightly lower. The per cent yield of carvone from oil of Spearmint may vary from 35 to 66 per cent. The per cent yield of carvone from the above samples exceeds these limits, running between 69.6 and 81.8 per cent.

To further establish the identity of carvone in this oil the separated ketone has been compared with carvone from a known source with reference to specific gravity, refractive index, optical rotation and other physical characteristics such as odor and appearance. The following table shows the relationship between the constants of the carvone in the Chrysanthemum oil as determined in this study and the constants which are given for carvone by Gildemeister (4):

TABLE III.

	Carvone from Oil of *Chrysanthemum Balsamita* var. *tanacetoides.*	Oil of Spearmint.
Specific gravity at 15° C.	0.9669	0.9652
Refractive index at 20° C.	1.4990	1.4988
Specific rotation at 20° C.	−59.20°	−59.66°

In addition to this the melting points of carvoximes prepared from Chrysanthemum oil and from oil of Spearmint have been determined and are found to be identical. The melting point of carvoxime was stated by Mulliken (5) to be 72° C. Melting point found in these determinations was 72° C. The melting point of mixed samples of carvoximes also was found to be 72° C.

As additional proof for the identity of carvone the dinitrophenylhydrazone of the Costmary oil has been prepared and the melting point found to be 186° C. to 187.5° C. as compared to 189° C. as cited by Kamm (3). The dinitrophenylhydrazone of carvone from oil of Spearmint was found to have a melting point of 189° C. It is felt, however, that with adequate purification, which has not been possible up to the present time, the two melting points will be found to check more closely.

SUMMARY.

The morphological characteristics of the plant, *Chrysanthemum Balsamita* L. var. *tanacetoides* Boiss. have been listed and the histology of the various plant parts has been described and illustrated.

Determinations of specific gravity, refractive index, optical rotation and percentage yield have been made on the volatile oil obtained from the plant. These have been compared with similar constants for oil of Spearmint.

The chief constituent of the oil has been isolated and identified as *l*-carvone by its physical constants, by the melting point of its oxime and by the melting point of the 2,4-dinitrophenylhydrazone.

REFERENCES.

(1) "Pharmacopœia of the United States of America," Lippincott, Philadelphia, 10th Revision, 1926.

(2) Jenkins, G. L., and DuMez, A. G., "Quantitative Pharmaceutical Chemistry," McGraw-Hill, New York, 1st Edition, 271 (1931).

(3) Kamm, O., "Qualitative Organic Analysis," John Wiley & Sons, New York, 2nd Edition, 170 (1932).

(4) Gildemeister, E., "The Volatile Oils," John Wiley & Sons, New York, 2nd Edition, Vol. 1, 439–443 (1913).

(5) Mulliken, S. P., "A Method for the Identification of Pure Organic Compounds," John Wiley & Sons, New York, Vol. 1, 218 (1911).

(6) "Allen's Commercial Organic Analysis," P. Blakiston's Son & Co., Philadelphia, 4th Edition, Vol. 4 (1911).

(7) Bailey, L. H., "Cyclopedia of American Horticulture," MacMillan, New York, Vol. 1, 312 (1910).

(8) Bailey, L. H., "Manual of Cultivated Plants," MacMillan, New York, 760 (1925).

(9) Bailey, L. H., and Bailey, E. Z., "Hortus," MacMillan, New York, 147 (1930).

(10) Britton, N. L., and Brown, A., "Illustrated Flora of the Northern United States, Canada and the British Possessions," Charles Scribner's Sons, New York, 2nd Edition, Vol. 3, 519 (1913).

(11) Chamberlain, C. J., "Methods in Plant Histology," University of Chicago Press, Chicago, 4th Edition, 48 (1930).

(12) Eames, A. J., and MacDaniels, L. H., "An Introduction to Plant Anatomy," McGraw-Hill Book Co., New York (1935).

(13) Fox, H. M., "Gardening with Herbs," MacMillan Co., New York, 128 (1933).

(14) Fuller, H. C., "Chemistry and Analysis of Drugs and Medicines," John Wiley & Sons, New York, 607 (1920).

(15) Gildemeister, E., "The Volatile Oils," John Wiley & Sons, New York, 2nd Edition, Vol. 3, 554 (1922).

(16) Green, Thomas, "The Universal Herbal," Caxton Press, London, Vol. 2, 647 (1824).

(17) Grieve, M., "A Modern Herbal," Cape, London, Vol. 1, 226 (1931).

(18) Hare, H. A., Caspari, C., and Rusby, H. H., "National Standard Dispensatory," Lea and Febiger, Philadelphia, 1085 (1916).

(19) Hegi, G., "Illustrierte Flora von Mittel-Europa," Lehmann, München. Bd., Hälfte, 2, 599–600 (1906).

(20) Hoffmann, O., "Compositæ" in "Die Naturlichen Pflanzefamilien," Engler, A., and Prantl, K., Teil IV, Abteil 4–5, 277–278 (1889).

(21) "Index Kewensis," Oxford Press, Fasc. 1, 526 (1893).

(22) *Ibid.*, Oxford Press, Fasc. 4, 1034 (1895).

(23) Linnæus, Carolus, "Species Planetarium," Tomus 2, 845 (1753).

(24) Mulliken, S. P., "A Method for the Identification of Pure Organic Compounds," John Wiley & Sons, New York, Vol. 2, 276 (1911).

(25) Parry, E. J., "The Chemistry of Essential Oils and Artificial Perfumes," Scott, Greenwood & Son, London, 4th Edition, Vol. 1, 237 (1921).

(26) Parry, E. J., "The Chemistry of Essential Oils and Artificial Perfumes," Scott, Greenwood & Son, London, 2nd Edition, 295 (1908).

(27) Parry, E. J., "The Chemistry of Essential Oils and Artificial Perfumes," Scott, Greenwood & Son, London, 4th Edition, Vol. 2, 230 (1922).

(28) "Pharmacopœia of the United States of America," Mack Printing Co., Easton, Pa., 11th Revision, 1936.

(29) Robinson, B. L., and Fernald, M. L., "Gray's New Manual of Botany," American Book Co., New York, 7th Edition, 847 (1906).

(30) Solereder, H., "Systematic Anatomy of the Dictyledons," Oxford, Vol. 1, 456 (1908).

(31) "Webster's Collegiate Dictionary," G. and C. Merriam Co., Springfield, 3rd Edition, 231 (1924).

A MODIFIED REDDISH CUP TECHNIQUE FOR EVALUATING THE GERMICIDAL ACTIVITY OF "LIQUID ANTISEPTICS."*

BY ARTHUR H. BRYAN.[1]

DISCUSSION.

Comparative test determinations of the bactericidal activity of antiseptics usually present difficulties in the laboratory because so many variable physical and bacterial growth factors tend to influence the results. Phenol coefficient tests based upon the time and concentration of any antiseptic to kill eighteen to twenty-four-hour cultures of the E. typhi are still used where antiseptic strength is required, but the results are subject to deviations or errors, and by many authorities are not considered very reliable. A check test using an entirely different determinative technique would therefore appear advisable. Recently Reddish evolved a method for testing antiseptic and germicidal ointments by the cup and smear method in which ointments were dropped into a cup, cut into plated culture media and seeded

* Presented before the Scientific Section, A. Ph. A., New York meeting, 1937.

[1] Science Department, Baltimore City College, Baltimore, Md.