breeding work. The plants are of a new variety resistant to the downy mildew disease, which is threatening the hop-growing industry of the Willamette valley.

EXCEPTIONAL OPPORTUNITIES AND POSSIBILITIES FOR DEVELOPMENT OF FUTURE CRUDE DRUG SUPPLY.

The importance of this work has been greatly emphasized in recent years by our dependence upon European countries for crude drugs and by the rapid disappearance of our native medicinal plants. At present, it is impossible to give adequate information for the successful cultivation of even some of our native medicinal plants, which grow wild about us. Hence, the experimental cultivation of plants is imperative for the future supplies of the essential crude drugs.

Many universities and institutions have begun to cultivate medicinal plants with reference to the economic aspect. The possibility of cultivating some of the existing wild medicinal plants on a commercial basis should be of vital interest and importance to all who have the drug industry and its future development at heart.

The development of the natural plant resources of the Pacific Northwest will lead toward a great future industry for which nature has prepared the foundations, with exceptional and superior natural advantages, as soil and climate.

The author is confident that the Northwest section of the country could eventually become the leading producer of crude drugs.

INFLUENCE OF PERIOD OF VEGETATION AND DEVELOPMENT OF PLANT ON THE ALKALOIDAL CONTENT OF HYOSCYAMUS NIGER L.*

BY ZDENEK F. KLAN.[1]

A study is here presented of the alkaloidal content of the different parts of this important drug plant during the various stages of its growth and development. Extensive tables are given showing the quantitative alkaloidal content with a qualitative differentiation as to whether it is chiefly atropine, hyoscyamine, scopolamine, tropine or scopoline.

Among many valuable conclusions it was found that (1) with the growth of the germinating plant the quantity of alkaloids in its organs decreases and that (2) in the order of their alkaloidal content the parts of the plant are as follows: root (both of annual and biennial plants), flowering tops, fruits, leaves and stems.

INTRODUCTION.

Literature records many studies dealing with the observations on alkaloids in pharmacologically important *Solanaceæ*, of which the chief subject of research has been *Atropa Belladonna* and in the second place *Datura Stramonium*. The authors have given very little attention to the not less important species *Hyoscyamus niger*. The majority of published investigations refer mostly to the quantitative determinations of alkaloids of commercial drugs official in pharmacopœias in order to ascertain their value, the greater number deal with histochemical observations of the localization of alkaloids in tissue, with the influence of selection on the alkaloidal content with quantitative determination of alkaloids in in-

* Scientific Section, A. PH. A., Miami meeting, 1931.

[1] Former holder of fellowship of the Rockefeller Foundation in food and drug control, Praha, Czechoslovakia.

dividual organs of the plant in various stages of the period of vegetation, etc. Only a few works pay attention to the qualitative histochemical determinations of alkaloids in the organs of *Solanaceæ* and to the observations of influence of various factors on their qualitative alkaloidal content.

In this study I have endeavored to ascertain not only the quantitative changes of alkaloids taking place in individual organs of the plant or their parts, during their development and during the period of vegetation, but ascertain also the changes which occur in the quality of alkaloids and their mutual relation.

In order to secure sufficient quantity of reliable material to be examined, on which the proposed observations could be performed, I founded for that purpose an experimental culture in quite a large garden in Liben—a suburb of Praha. The fact must be mentioned that the garden is exposed all day to the sun. The seeds of wild growing plants, both annual and biennial, which have been used for sowing, have been gathered from dead herbs in the winter season. The garden soil, where the experimental culture was established, has not been fertilized for a number of years. The material to be examined has been gathered in all cases at about nine o'clock in the morning of sunny days after bright nights and, immediately after gathering, it has been dried in a previously warmed drying vacuum apparatus, heated by means of steam at a temperature of 40° C.

For the quantitative macrochemical determinations of alkaloids the official method of the U. S. P. X has been employed. In some cases, where it was impracticable to secure prescribed quantity of material, it was necessary to assay smaller or quite small quantity.

The qualitative and proximate quantitative histochemical examinations have been carried out according to the method of Klein and Klein-Sonnleitner[1] by the use of microextraction method only, which during the assaying has proved much more satisfactory than the vacuum micro-sublimation method. The proximate quantitative results obtained by this mentioned histochemical method are interpreted in "γ" similarly after Klein-Sonnleitner as follows:

ø	no reaction obtained
+	1 γ of alkaloids or less
++	2 γ of alkaloids or less
+++	5–20 γ of alkaloids or less
++++	50–100 γ of alkaloids or less
+++++	100 γ of alkaloids and more

From the microchemical reagents for the qualitative determination of alkaloids have been employed $N/10$ iodine volumetric solution, picrolonic acid, gold chloride (5%) and strontium iodide plus mercuric chloride solution, reactions of which offer with the mydriatic alkaloids of *Solanaceæ* characteristic precipitates identifying the alkaloids even in their mixtures.

EXPERIMENTAL.

The material, employed for both quantitative and qualitative determinations, has been chosen and gathered in a manner so that I could ascertain not only the influence of various stages of the period of vegetation on the alkaloidal content of

[1] I advise the readers to consult the original papers.

individual organs or their parts but also their content of alkaloids during various stages of development.

I began the experiments with germinating plants during the time when they fully developed both of the cotyledons but have not shown as yet any apparent marks of leave protuberance; their roots were very tender and showed primary tissue with diarch fibrovascular bundles. As a further stage of development young plants have been taken, in which from the leave protuberance minute leaves had already developed. In examining alkaloids in the root of young plants bearing first 2–6 leaves there have been taken into consideration four various successive stages of development, which have been considered after the changes occurring in the tissue: *a* stage, when the parenchyma cells neighboring the inner part of sieves divide by the tangential walls to produce cambium; *b* stage, when cambium formed on the outer side tissue of secondary bark and on the inner side tissue of secondary wood; *c* stage, when the rhizodermal cells of the root are entirely withered and when the parenchymatic exodermal layer of cells obliterated and finally, *d* stage, when the root tissue prevails over the secondary formed tissue and when the meristematic cork layer has been developed.

For the determination of alkaloids in the leaves of young tender plants there have been investigated leaves of the size of cotyledons and leaves of a length of 2 cm. Similarly the epicotyl axil part, two stages of development, have been taken into consideration. These are: the stage when the epicotyl has attained the length of about 2 mm., in which time its anatomical structure consisted still of primary tissue and then the stage when epicotyl has formed a stem of about 2 mm. thick, in which was apparent cambium of several layers, which had given origin to the secondary tissue at certain extent. In the hypocotyl has been examined the alkaloidal content of the germinating plant bearing first two small leaves and of the plant, where the leaves had attained a length of one cm.

In the adult plant the alkaloidal content has been determined first in the root of both the annual and the biennial plant of first and second year of growth and also in the hibernating root. The content of wood and bark has been determined separately in order to find out the comparative proportion of the amount of alkaloids of both parts of this organ. In order to ascertain the influence of various stages of the period of vegetation on the alkaloidal content of root, the roots of both varieties have been gathered in four subsequent months—June, July, August and September—in every case at the beginning of the month with exception of September, when the roots were not gathered until the end of the month. The roots of biennial plants in the second year of growth have been gathered also at the beginning of May. The wintering root of the biennial plant gathered in the same time has also been taken into consideration. To complete these observations the alkaloidal content of the primary root deprived of secondary roots has also been determined.

For the determination of alkaloidal content in the main axis, only the axis of the annual plant has been employed so that cuttings of the same length of about 10 cm. above the ground have been prepared. The thickness of the cuttings selected has always been about the same, that is, at the first gathering in May averaging about 4 mm. and at the second gathering, in September, about 10 mm. In the individual parts of the main axis, that is, in the bark wood and pith, the content

has been examined separately for the purpose of ascertaining its ratio in these individual parts of the axis. Further consideration has been taken of young slender twigs, older thicker twigs, basal portion of the main axis and the entire stem. All material for these determinations has been gathered in two periods, that is, first in June and then in September.

The examinations of the leaves have been performed on both annual and biennial plants; here the petiolate leaves of the rosette only, for which examinations various lengths have been chosen, that is, in both cases 4 cm., 10 cm. and 20 cm. The leaves have been gathered first in June and for the second time in September. Furthermore, the alkaloidal content of the midrib of sessile leaf of the annual plant has been determined in which determination the length of the leaf has been chosen—the same as in the examination of alkaloidal content of entire leaf and likewise has been determined the content of petiole and lamina of the leaves of rosettes of the same length as in other determinations. Furthermore, the alkaloidal content has been examined in the withering leaves which have been turning yellow and in the very yellow, almost dead leaves; for these determinations the leaves have been gathered at the beginning of October.

In the flower the alkaloidal content has been examined in every part and in respect to the fact, that after fertilization of ovules there takes place during the development of the future fruit considerable biogenetic change, which may influence also the quantity and quality of alkaloids, various stages of development of some parts of flower, later of fruit, have been taken into consideration. This concerns especially the seeds wherein, in the first place, white unripe seeds have been taken into consideration, then brownish colored unripe seeds and, finally ripe seeds; furthermore, also the alkaloidal content of ripe seeds which have been stored for several years has been investigated. Also, in the pericarp and in the entire fruit, three stages of development have been selected. The stages of development of other parts of the flower in which the investigation of alkaloids has been made and also the parts of the flower, where no attention has been paid to the special stages of development, are given in detail in the following table of individual investigations.

In order to complete the observations of alkaloidal content of individual organs or their parts and of the influence of various stages of the period of vegetation, the flowering tops have also been taken into consideration. The tops have been gathered in four stages, that is—in June, not yet flowering; in July, with fully developed flowers; in August, bearing young capsules and, in September, bearing only fruits, no flowers.

COMPARATIVE SUMMARY OF RESULTS OBTAINED IN THE INDIVIDUAL DETERMINATIONS.

Organ or Its Part.	Quantity in %.	Quality. Atropine.	Hyoscyamine.	Scopolamine.	Tropine.	Scopoline.
Germinating and Young Plants.						
Germinating plant with fully developed cotyledons	0.018	0	0	++	0	0
Young plants bearing first leaves	0.042	0	0	++	0	0
Rootlet of young plant:						
Stage *a*	0.048	0	0	++	0	0
Stage *b*	0.055	0	0	++	0	0

Stage *c*	0.061		0	0	++	0	0
Stage *d*	0.098		0	+	++	0	0
First pair of leaves of size of cotyledons	0.015		0	0	+	0	0
Leaves of length 2 cm	0.039		0	0	+	0	0
Epicotyl with primary anatomical structure	0.047		0	0	++	0	0
Epicotyl showing transition to secondary anatomical structure	0.038		0	0	+	0	0
Hypocotyl of germinating plant	0.045						
Hypocotyl of young plant bearing first leaves	0.027						
Root.							
Annual plant.							
June	0.103	0.155	0	++	+	0	0
July	0.127		0	+++	++	0	0
August	0.205		0	++++	+++	0	0
September	0.186		0	++++	++	0	0
Wood	0.073		0	++	+	0	0
Bark	0.290		0	+++	++	0	0
Biennial plant.							
(*a*) In the first year:							
June	0.093	0.167	0	++	+	0	0
July	0.147		0	+++	+++	0	0
August	0.196		0	+++	++	0	0
September	0.234		+	++++	+	0	0
Wood	0.062		0	++	0	0	0
Bark	0.305		0	+++	++	0	0
Wintering root in January	0.244		+	+++	++	0	0
(*b*) In the second year:							
May	0.116	0.176	0	++	+	0	0
June	0.144		0	+++	++	0	0
July	0.187		0	+++	++	0	0
August	0.236		0	+++	+++	0	0
September	0.197		+	+++	0	0	0
Wood	0.084		0	+++	0	0	0
Bark	0.312		0	++++	++	0	0
Primary root deprived of the secondary roots	0.082						
Secondary roots	0.111						
Stem.							
Main axis:							
Bark — May	0.032		0	+	0	0	0
Bark — September	0.020		0	+	0	0	0
Wood — May	0.010		0	0	0	0	0
Wood — September	Trace		0	0	0	0	0
Pith — May	0.065		0	+++	+	0	0
Pith — September	0.008		0	0	0	0	0
Basal portion of the main axis:							
June	0.048		0	++	0	0	0
September	0.010		0	0	0	0	0

Young slender twigs:						
June	0.119	0	+++	+	0	0
September	0.062	0	++	0	0	0
Older thicker twigs:						
June	0.069	0	++	0	0	0
September	0.012	0	0	0	0	0
Entire stem of annual plant:						
June	0.045	0	++	0	0	0
September	0.032	0	+	0	0	0
Leaves.						
Sessile leaves of annual plant.						
Length:						
4 cm. June	0.056	0	++	0	0	0
4 cm. September	0.049	0	++	0	0	0
10 cm. June	0.068	0	++	+	0	0
10 cm. September	0.021	0	+	0	0	0
20 cm. June	0.045	0	++	0	0	0
20 cm. September	0.009	0	0	0	0	0
Midrib:						
Length of the lamina:						
4 cm	0.081					
10 cm	0.113					
20 cm	0.102					
Basal leaves of the rosette.						
Length:						
4 cm. June	0.161	0	++	+	0	0
4 cm. September	0.053	0	++	0	0	0
10 cm. June	0.085	0	++	+	0	0
10 cm. September	0.032	0	+	0	0	0
20 cm. June	0.072	0	++	+	0	0
20 cm. September	0.018	0	0	0	0	0
Petiole.						
Length of the lamina:						
4 cm	0.076					
10 cm	0.126					
20 cm	0.115					
Lamina.						
Length:						
4 cm	0.065					
10 cm	0.079					
20 cm	0.042					
Yellowing leaves	0.030	+	+	0	0	+
Strongly yellow leaves	0	0	0	0	+	+
Flower.						
Peduncle	0.103	0	+++	+	0	0
Calyx	0.042	0	+	0	0	0

Corolla { Living	0.037	0	+	0	0	0
Corolla { Withering	Trace	0	0	0	+	0
Filaments of stamens:						
Of unripe anthers	0.030	0	+	0	0	0
Of ripe anthers	0.002	0	0	0	0	0
Anthers { Unripe	0.028	0	+	0	0	0
Anthers { Ripe	0.015	0	+	0	0	0
Pollen grains	0	0	+	0	0	0
Ovary deprived of ovules	0.075	0	++	+	0	0
Ovules	0.095	0	0	+++	0	0
Style with stigma	0.039	0	++	0	0	0
Fruit.						
Peduncle.						
Young fruit	0.051	0	++	0	0	0
Ripe fruit	0.023	0	+	+	0	0
Calyx.						
Young unripe fruit	0.029	0	+	0	0	0
Ripe fruit	Trace	0	0	0	0	0
Dry dead herb	0	0	0	0	0	0
Pericarp.						
Young fruit	0.063	0	+++	0	0	0
Adult fruit	0.045	0	++	0	0	0
Ripe fruit	0.025	0	+	0	0	0
Fruit with calyx and penduncle.						
Young	0.106					
Adult	0.098					
Ripe	0.025					
Tops.						
With no flowers, June	0.053	0	++	0	0	0
Flowering, July	0.074	0	++	+	0	0
Flowering with young fruits, August	0.092	0	++	++	0	0
With fruits, no flowers, September	0.089	0	+++	+	0	0
Seeds.						
Unripe, white	0.125	0	++	++++	0	0
Unripe, brownish	0.106	0	++	+++	0	0
Ripe	0.098	+	++	++	0	0
Stored for several years	0.054	+	+	0	0	+

From this table of results obtained in individual quantitative and qualitative macro- and microchemical examinations can easily be recognized, not only the comparative ratio of the content of alkaloids in the individual organ or its parts but also the variability of content in various stages of the period of vegetation, in regard to the quality and the quantity.

It is interesting that in the first stages of development of germinating plants and young plants bearing first leaves occur of the alkaloids only scopolamine, the amount of which increases during the growth of the plant. The increase of alkaloids is also to be noted in the rootlet, in which in the advanced stage of develop-

ment, when the secondary tissue begins to prevail over the primary tissue, appears also hyoscyamine. With advanced growth of other organs the content of scopolamine decreases, as can be noticed in the leaf, in epicotyl and in hypocotyl; therefore the total increase of alkaloidal content in young plants is due to the increasing alkaloidal content in the rootlet.

In the root of an adult plant a larger amount of alkaloids is to be found in the bark than in the wood, as in that of annual and in the biennial plant; in almost all cases there is hyoscyamine and scopolamine but hyoscyamine always is in predominating quantity; the third alkaloid, atropine, appears in a few instances.

Study of alkaloids in the bark and wood of the root of *Hyoscyamus niger* has been undertaken already by Molle, who through histochemical method proved their presence only in the bark tissue, and also Siim-Jensen, who found the largest amount of alkaloids in the medullary rays of the bark tissue and then in periderm with exception of the cork layer. Sievers, on the contrary, states regarding *Atropa Belladonna*, that it is not always a rule that the bark of the root is a larger bearer of alkaloids than the wood. In young roots of *Datura Stramonium* Molle found the alkaloids chiefly in the wood, whereas in the bark there was only a very small amount of alkaloids; in older roots he found the alkaloids also in the medullary rays, and he was of the opinion, that through these the alkaloids migrate into the outer layers, that is, in the cortex, where they then prevail. There is no marked difference between the alkaloidal content of the root of annual and biennial plant in the first and in the second year of growth, though the root of the biennial plant in the second year shows slightly higher content. As to the influence of the age of the plant on the alkaloidal content of the root there are given in the literature many quantitative notes concerning chiefly the *Atropa Belladonna*, which, however, have for our study no special comparative significance although we are dealing with a species closely related, because this plant is a perennial one and consequently its biological processes proceed under different conditions. Only that much, perhaps, is to be said that according to the investigations of Gerrard, Lefort, Trögele, Burman and Sievers it is hardly possible to deduct a rule, according to which with the advancement of the age of the plant the fluctuation of the alkaloidal content of the root should conform.

The content of alkaloids in the root increases with the advancing growth of the plant until a definite time, after which it again decreases. In the annual and biennial plant of the second year the alkaloidal content in the root has the highest grade during the time when the plant is in full blossom and when it bears some fully developed capsules. In my investigations the alkaloidal content of the root of the annual plant reached its highest limit earlier than the root of the biennial plant. In the root of the biennial plant in the first year of growth the alkaloidal content rises continually until the time, when the rosette of leaves is dying off; in the hibernating root in the winter time there appears a slight decrease whereas at the beginning of the second period of vegetation it decreases very remarkably. The dead root does not contain any alkaloids.

Literature gives many investigations about the influence of the period of vegetation on the alkaloidal content of the root. From the pharmacognostically important *Solanaceæ* the attention has been hitherto devoted mostly to the *Atropa Belladonna*, less to *Datura Stramonium* and least to *Hyoscyamus niger*.

The alkaloidal content in the root of *Hyoscyamus niger* was investigated first by Thorey and Dragendorff. Dragendorff found that the content of alkaloids of the root, in the time when there appear on the plant the first flowers, is much higher than in the root of fully flowering plant. Squire states that the root of biennial plant is in the early spring, in the time when the plants begin to show first marks of new life, much richer in alkaloids than the root of the same locality gathered in September in the previous year. My finding, therefore, is not in conformance with the statement of Dragendorff nor with the statement of Squire. In connection with the increase of alkaloidal content in the root of the biennial plant the opinion of Buddel is interesting. He presumes the increasing of alkaloids in the plant as being in certain relation to the increase of starch in the tissue, and he explains this opinion by the fact, that in the spring, when the plant does not contain starch, it is poor in alkaloids, whereas in the autumn, when starch is found in larger amounts, it is richer in alkaloids.

In the root of both annual and biennial plants both hyoscyamine and scopolamine are present during most of the period of vegetation, but scopolamine always in smaller amount; with the increasing content of hyoscyamine increases also the content of scopolamine. In the root of biennial plants the occurrence of atropine is striking, which appears, however, only in a minute amount but always at a definite time; that is, at the beginning of autumn and in the hibernating root in winter time.

In the stem the largest amount of alkaloids appears in the pith, but only in the springtime, when the pith is still fully preserved. With the growth of the stem, with which the pith tissue gradually dies off, decreases also the content of alkaloids until autumn when it is limited to very minute amounts. The wood portion of the stem contains less alkaloids than the bark portion. With the progress of the period of vegetation toward autumn the amount of alkaloids in the stem decreases. Sievers found in the stem of *Atropa Belladonna* similar conditions and according to his investigation the difference of the alkaloidal content of thin and thick twigs is still more distinct. The alkaloids in the main axis as in the branches consist almost entirely of hyoscyamine; scopolamine appears only in small amounts solely in young and slender twigs in the springtime, when these show high alkaloidal content.

With growth of the leaf the amount of alkaloids increases to a definite limit and then decreases again, although this decrease and previous increase in adult leaves is not very definite in comparison with the increase of alkaloidal content in the youngest minute leaves. Concerning the ratio of alkaloidal content of individual parts of the leaf, the midrib and the petiole are always richer in alkaloids than the lamina. Alkaloidal content of the leaves of the rosette is, according to my investigations, given herein, higher than the content of leaves of the annual plant. In this respect there are different opinions. Wehmer states that the leaves of biennial plants are poorer in alkaloids than the leaves of annual plants. Kreyer finds in annual plants the same alkaloidal content as in the biennial plants, but does not indicate at what time and under what conditions and circumstances, in both cases, the leaves have been gathered. Some time ago I (Klan) found that the leaves of annual plants show lower alkaloidal content though not more marked than the leaves of biennial plants of first and second years of growth and that the content of last mentioned variety is in the first and second year, with insignificant fluctuations, almost the same. The leaves are always richer in alkaloids in the

springtime than in the fall and the highest alkaloidal content shows in the flowering-time in the summer months, when the greater or smaller fluctuation remains in average about the same height until the end of the period of vegetation when it begins to decrease. Similar results were obtained also by Pater and on the *Atropa Belladonna* by Gerrard, Trögele, Sievers, Bauer and Kreyer. On the other side Thorey and after him Dragendorff state, that according to their investigations, the leaves of *Hyoscyamus niger* contain larger amount of alkaloids before the flowering-time than after it. Dragendorff gathered the leaves at the same locality at the beginning of June and beginning of July and found, that in the first case the alkaloid content amounted to 0.058%, in the second case, however, only to 0.015%. Consequently my investigations are not in accord with these statements. In the dying off yellowing leaves the alkaloids rapidly decrease and finally disappear. Between hyoscyamine and scopolamine we find in the leaves a similar relation as in the root, it means that with the increase of total content of alkaloids increases also the content of scopolamine, but scopolamine remains always in lesser amount. Where there is low content of hyoscyamine, scopolamine does not occur. In the dying yellowing leaves appears also a small quantity of atropine and besides this the basic decomposition products of alkaloids concerned, that is tropine and scopoline, the appearance of which is to be seen in the post-mortem autolytic and fermentative action of the cell content.

From the parts of flower the greatest alkaloidal content, with exception of the peduncle, falls upon the ovules. It is interesting that in these hyoscyamine does not appear together with scopolamine as in other organs but only scopolamine; there is here, therefore, a case similar to the occurrence of alkaloids in germinating plants with primary anatomical structure. On the other hand most of the flower parts—that is, filaments of the stamens, anthers, pollen grains and style with stigma—contain only hyoscyamine and it is only in the walls of the ovary, that there appears besides hyoscyamine also scopolamine, but in smaller quantity. After the fertilization of the ovule during the development of the fruit the alkaloidal content of all parts of the flower, respectively, of the fruit, decreases with exception of ovules which later develop in seeds, whereas in the unripe seeds higher alkaloidal content is found than in the ovules, although the content is insignificant, in comparison with the decrease of alkaloidal content of other parts of the fruit. As it was mentioned, in the ovules there occurs of the alkaloids only scopolamine but after fertilization of ovules during the development of the seed also hyoscyamine begins to appear and in the time of full ripeness of the seeds it equals the quantity of both of these alkaloids. In entirely ripe seeds it is then possible to ascertain also a small amount of atropine. In the seeds which have been stored for a long time scopolamine disappears and in its place appears its basic decomposition product, scopoline.

If we look over the results of quantitative determinations, we find that on the young organs falls relatively a much higher content of alkaloids than on the older, or those which are withering. Consequently the flowering tops which are composed of the developing organs are first carriers of alkaloids. Newcomb and Haynes actually found in the flowering tops of *Hyoscyamus niger* higher alkaloidal content than in leaves; Goddijn reports similar findings in his fenotypes, where in successive order of alkaloidal content he places the flowering tops in the second place. He puts the roots in first place, the stem in third and finally the leaves. Gerrard

on the contrary does not find any marked difference in the alkaloidal content of flowering tops and leaves. From the results of my experiments it can be deducted that the tops, which do not bear the flowers as yet are poorer in alkaloids than the flowering tops, in which the alkaloidal content during the period of vegetation increases very markedly until the autumn, when it begins to decrease. The behavior of the tops is determined by the increase of alkaloids during the period of vegetation as in the case of the leaves which, as can be seen from the table of results, are poorer in alkaloids than the tops. The results obtained by investigations of my own confirm, therefore, the statements of Newcomb and Haynes and of Goddijn; they do not, however, agree with the statements of Gerrard.

COMMENTS.

Comparing the alkaloidal content of adult and young plants, it is necessary to take into consideration the fact, that in the adult organs, in most cases, there prevails the secondary formed tissue, which is free from alkaloids as is the case, especially, in tracheæ, sieves and sclerenchyma cells, which the weight of dried material makes markedly heavier.

The occurrence of atropine in the root of biennial plants should not be seen in the racemization of hyoscyamine due to the autolytic and fermentative action during drying, because all of the experimental material was dried under the same conditions. If it would be supposed that during the time of drying occurred the racemization of hyoscyamine in atropine, then consequently the atropine should have been found also in other experimental material in various quantities, according to the grade of influence of fermentative or autolytic post-mortem action. Relative to its casual occurrence in the root there is the opposition also of the circumstance, that it has been found in decline during the period of vegetation, and in the winter time, that is in the time when the root stores the reserve matters, in connection with which its occurrence could be explained. The occurrence of atropine and of decomposition products—tropine and scopoline—in the withering leaves could be accounted for in the influence of fermentative and autolytic action. The formation of atropine in the ripe seeds was probably due to the biogenetic processes which take place during their development. The occurrence of scopoline in the seeds which have been stored for a long time could, perhaps, be explained by the influence of the decomposition of the fixed oil contained in the seeds.

CONCLUSIONS.

The quantity of alkaloids decreases in the organs during the growth of the germinating plant.

The root of both annual and biennial plant shows the highest alkaloidal content before the decline of the period of vegetation in the last summer months. The secondary roots are richer in alkaloids than the primary root. The bark of the root contains a larger quantity of alkaloids than the wood.

The branches are richer in alkaloids than the main axis and their alkaloidal content is higher at the beginning of the period of vegetation than at its decline.

The alkaloids increase in the leaves with their growth up to a definite period and afterward decrease. At the beginning of the flowering-time the leaf has the

highest alkaloidal content. The lamina is poorer in alkaloids than the midrib and petiole. In the withering leaf the alkaloids are disappearing very rapidly.

The alkaloids disappear in the calyx, corolla, stamens, pericarp and style as soon as these parts of the flower cease to function. Most of the alkaloids are contained in the unripe pericarp. The ovules are very rich in alkaloids. After the fertilization of the ovule the quantity of alkaloids increases in the young seed almost until its full ripeness, when it decreases slightly.

The young unripe fruit is richer in alkaloids than the ripe fruit.

The flowering tops contain a larger quantity of alkaloids than the non-flowering tops.

The dead organs do not contain any alkaloids.

In order of alkaloidal content there follow in succession the root both of annual and biennial plants, flowering tops, fruits, leaves and stem.

In the organs of primary stage of development there occurs only scopolamine and hyoscyamine in the later more advanced stages. This is true especially with the germinating plant and ovules.

In all organs which have passed the primary stage of development there predominates quantitatively without exception, hyoscyamine and scopolamine, the latter always in smaller quantity, if it occurs at all. The increasing content of hyoscyamine also increases the content of scopolamine.

Atropine occurs only in the root of the biennial plant at the end of the period of vegetation and in the wintering root, in the ripe seeds and in the withering leaves where its appearance is post-mortal.

Tropine and scopoline occurring in the withering organs are produced by the decomposition of alkaloids which is due probably to the autolytic and fermentative action.

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STUDIES ON THE DETERMINATION OF CAMPHOR IN CAMPHOR LINIMENT.

II. U. S. P. X Method for Liniments Made with Oils Other Than Cottonseed.*

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INTRODUCTION.

In a previous communication (1), in which the literature was reviewed, the U. S. P. method for the determination of camphor in the official camphor liniment was studied. It was found that the method was not sufficiently accurate for the determination of camphor in this preparation. When 20 per cent liniments were assayed, results were found to be as much as 1.34 per cent low. The error was due to the oxidation of the double bond in the olein present in the oil with the probable formation of an enol compound and lower esters and acids.

The official liniment of the U. S. Pharmacopœia is made with a base of cottonseed oil. However, the use of other oils, in this country and abroad, has been practiced in the manufacture of this preparation.

The purpose of the investigation reported in this paper was to determine the applicability of the U. S. P. X method to liniments made with oils other than cottonseed.

MATERIALS AND PROCEDURE.

Different fixed oils, including almond, corn, olive, peanut, rape, sesame and sunflower, were used for the preparation of different camphor liniments. The purification of the camphor and the preparation of the samples were accomplished by the methods described in a previous paper (1). The method of determination was that described in the U. S. Pharmacopœia X, the details of which were described in the communication referred to above.

EXPERIMENTAL.

In Table I is shown the effect of heating in a constant-temperature air oven, one sample of each of the oils. The percentages shown are calculated from the gain in weight of the oil due to the oxidation during heating. This gain, of course, would account for most of the error found when camphor was determined by this

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